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Studies on duck plague

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INTRODUCTION

Duck plague (duck virus enteritis) is an acute contagious herpes viral disease of Anatidae (ducks, geese, and swans). Baudet (1923) first described an outbreak in the Netherlands and thought the virus was a duck-adapted strain of fowl plague. Subsequent epidemics have been reported in France (Lucam, 1949), China (Jansen and Kunst, 1964), Belgium (Devos et al., 1964), India (Mukerji et al., 1963a, 1963b, 1965), Britain (Hall and Simmons, 1972), and the United States (Leibovitz and Hwang, 1968a, 1968b; Snyder et al., 1973). These outbreaks occurred in domestic flocks, and the importance of duck plague in free-flying waterfowl was not determined.

In January and February of 1973, the first major epidemic of duck plague among free-flying waterfowl occurred at Lake Andes, South Dakota. An estimated 42% of 100,000 mallard ducks (Anas platyrhynchos) and 3% of 9,000 Canada geese (Branta canadensis) wintering in the area died. The high mortality among mallard ducks was unusual since these ducks had been reported to be more resistant than other ducks to the virus (Van Dorssen and Kunst, 1955). Leibovitz (1972) stated that "the specific pathologic response to duck plague virus is dependent upon the species affected; age, sex and susceptibility of the affected host; stage of infection; and virulence and intensity of virus exposure." At Lake Andes

the waterfowl were concentrated on a small lake during cold weather, and several factors may have contributed to the epidemic.

Before an effective disease control program can be established, a knowledge of contributing environmental factors and disease pathogenesis is necessary. Environmental factors play an important role in several diseases such as pasteurellosis of cattle and the formation of human oral herpes simplex lesions.

Little is known about the effect of low levels of environmental lead on diseases, but low levels of lead have been reported to increase the susceptibility of mice to viral diseases (Gainer, 1973) and Salmonella (Hemphill et al., 1971). Lead is abundant in the duck environment. A large number of lead shot are shot into lakes and rivers by hunters each year. These lead shot are ingested by ducks while they are feeding in shallow water. The shot lodge in the ventriculus and serve as grit. During the process lead shot are broken up, and lead is absorbed by the duck's digestive tract. Bacteria of an ecosystem are commonly found as secondary invaders in chronic viral diseases, but their significance in duck plague has not been determined. Dardiri (1971) reported that mortality was greater in flocks where Salmonella and Pasteurella were also present.

This study was conducted with the three following objectives: (1) to describe the cytopathology produced by duck plague virus isolated from Lake Andes in laboratory ducks, (2) to determine if low levels of lead increase the susceptibility of ducks to duck plague virus, (3) to determine if bacteria play a synergistic role in production of lesions ascribed to duck plague.

LITERATURE REVIEW

Duck plague is an acute contagious disease of the family Anatidae (ducks, geese, and swans). Baudet (1923) reported an acute hemorrhagic disease of ducks produced by a filterable virus in the Netherlands. Chickens were not susceptible to the virus, and he concluded the disease was caused by a duck-adapted strain of fowl plague virus. DeZeeuw (1930) reported on a similar outbreak and also concluded the disease was initiated by a duck-adapted strain of fowl plague virus. Bos (1942) concluded the disease was not fowl plague because of its specificity for ducks and was the first to use the term duck plague. Other outbreaks have been reported in France (Lucam, 1949), China (Jansen and Kunst, 1964), Belgium (Devos et al., 1964), India (Mukerji et al., 1963a, 1963b, 1965), Britain (Hall and Simmons, 1972), and the United States (Leibovitz and Hwang, 1968a).

The susceptibility of domestic and wild ducks varies from species to species. The disease has been reported in the mallard duck (Anas platyrhynchos platyrhynchos), black duck (Anas rubripes), Canada goose (Branta canadensis), bufflehead (Bucephala albeola), greater scaup (Aythya marila), mute swan (Cygnus olor), Muscovy duck (Cairina moschata), Egyptian goose (Alopochen aegyptiacus), Garganey teal (Anas querquedula), gadwall (Anas strepera), European widgeon (Anas penelope), wood duck (Aix sponsa), shoveler (Spatula clypeata),

sheldrake (Tadorna tadorna), tufted duck (Aythya fuligula), common eider (Somateria mollissima), white-fronted goose (Anser albifrons), and bean goose (Anser fabalis). Duck plague has also been reported in a variety of domestic ducks (Anas platyrhynchos domesticus) including White Pekin, Khaki Campbell, and Indian Runner, and in domestic geese (Anser anser domesticus). In contrast European teal (Anas crecca), pintail (Anas acuta), and Grey Call duck (miniature domesticated mallard duck, Anas platyrhynchos platyrhynchos) are reported to be resistant to doses of duck plague virus that are lethal to other species of ducks; however, they did produce antibodies against the virus after experimental exposure. Mallard ducks were more resistant to infection and have been considered as a possible reservoir of infection (Van Dorssen and Kunst, 1955).

Since duck plague virus can be adapted to grow in chicken embryos and chickens up to 2 weeks of age (Jansen, 1964b), it has been suggested that the virus has a wide host range. This has not been proven experimentally since most other birds, for example the hen and pigeon (Jansen, 1961), cannot be experimentally infected. Herring gulls (Larus argentatus) and black-headed gulls (Larus ridibundus) were resistant to experimental infections and did not develop antibodies against the virus (Jansen, 1964a).

Epidemiologic studies favor the theory that duck plague virus entered the United States in imported exotic Anseriformes. The virus also may have entered the United States in carrier wild waterfowl which migrated north in the summertime from endemic areas in Europe and then returned south along the eastern Atlantic flyway. Dutch nationals who were employed on New York duck farms may have also mechanically transmitted the virus (Newcomb, 1968). In 1967 Dardiri and Hess examined serum from wild ducks and geese to determine the prevalence of duck plague in the wildlife population. Only 3 of 2,936 samples examined had diagnostically significant antibody titers of 1.75 log 10 or greater. All three of the samples were obtained from birds in the New England area. Ducks from the other flyways in the United States were negative for antibodies against duck plague virus. From these serological results, it was concluded that duck plague virus had recently entered this country (Dardiri and Hess, 1968b). In a similar serological survey in domestic ducks following an epidemic, 33% to 60% of the surviving ducks had virus neutralization titers between 1.75 and 3.0 log 10 and greater (Dardiri and Hess, 1968b). The conclusion that duck plague recently entered the United States may be questioned because Asplin (1970) concluded that the exposure of wild waterfowl was low in the Netherlands after a serological survey of wild waterfowl in an endemic area. The significance of the disease in

wild waterfowl has not been determined; reported serological results do not suggest it is an important disease of wild ducks and geese (Leibovitz, 1968; Leibovitz and Hwang, 1968a). The greatest reported incidence of duck plague in wild Anseriformes occurred during normal migratory periods and indicated that susceptible ducks were moving into an infected environment. This could also represent aggregation of carrier and susceptible birds (Leibovitz, 1968).

Virus

Most of the early work with duck plague virus was conducted to differentiate it from Newcastle disease virus and fowl plague virus (Dardiri and Hess, 1968a; Jansen, 1951, 1961). Two strains, the old (O) strain and Wageninger (W) strain, have been found which are antigenically identical; but the 'O' strain is more virulent than the 'W' strain (Jansen, 1961). The virus differs from Newcastle disease virus in being nonhemagglutinating (Jansen, 1961) and nonhemadsorbing (Dardiri and Hess, 1968a). Plaques are formed in tissue culture, and intranuclear inclusion bodies are present in tissue culture cells (Dardiri and Hess, 1968a).

Viral particle size has been determined by filtration and by electron microscopic studies. Infective particles passed filters greater than 100 nm but less than 220 nm in diameter

(Hess and Dardiri, 1968). Using the electron microscope, intranuclear forms of the virus were found to be 91 nm in diameter with a dense core 48 nm in diameter. Cytoplasmic forms obtained an envelope as they left the nucleus and were approximately 181 nm in diameter with a 75 nm core (Breese and Dardiri, 1968). Characteristics such as size, presence of an envelope, and the formation of inclusion bodies indicate duck plague virus is a herpesvirus (Breese and Dardiri, 1968) although the capsid structure has not been described.

The resistance of duck plague virus to some chemical and physical agents has been determined. Virions are sensitive to chloroform and ether; and exposure to trypsin, chymotrypsin, and pancreatic enzymes markedly reduces their infectivity (Leibovitz, 1972). Heating particles at 56° C for 10 minutes or 50° C for 120 minutes will destroy the virus. If the virus is left at room temperature, it is not inactivated until after 30 days. In contrast, drying over calcium chloride at 22° C for 9 days destroys the infectivity of the particles. The virus is viable at pH 7 through 9 over a 6-hour period, but a measurable reduction in viral titer is noted at pH 5, 6, or 10. Solutions with a pH of 11 or greater and a pH of 3 or less rapidly destroy duck plague virus (Hess and Dardiri, 1968).

Viral replication has been studied in cell culture. Particles of virus first appear in the nucleus 2 hours after inoculation. At 12 hours definite developmental forms of

virus without envelopes and a dense core are first detected in the nucleus. Enveloped morphologically complete forms are present in the cytoplasm 24 hours post-inoculation. Cell-associated viral titers in the cultures increase 0.5 log units every 2 hours up to 8 hours at which time extracellular particles are detected. A maximum cell-associated titer is reached 48 hours post-inoculation, and the maximum extra-cellular titer is reached 60 hours post-inoculation (Breese and Dardiri, 1968).

Culture

Primary isolation of duck plague virus can best be obtained on the chorioallantoic membranes of 9- to 14-day-old embryonated duck eggs (Newcomb, 1968). Maximum death in the duck embryos occurs 120-192 hours after inoculation. Higher virus concentrations are present in chorioallantoic membrane suspension than in the embryo or yolk sac (Butterfield et al., 1969). Field isolates will not grow on the chorioallantoic membranes of chicken embryos until after they have been passed through duck embryo tissue cultures five times. Cytopathogenicity for cell culture increases for the first five passages in duck embryo fibroblasts. After five passages the virus is sufficiently adapted to then grow in chicken embryo cell culture, but the virus is still virulent for ducks. The titer of field isolates of duck plague virus increases with each

subsequent passage in cell culture (Dardiri, 1969). Cytopathogenicity in cell cultures is characterized by pyknosis and shrinkage and lysis of affected cells. After the second passage, intranuclear inclusion bodies are seen in the fibroblasts as early as 12 hours post-inoculation (Dardiri, 1969). Further passage of the virus in chicken embryos attenuates the virus (Dardiri, 1969).

Transmission

Experimentally ducks can be infected by oral, intranasal, intraperitoneal, intramuscular, and cloacal inoculation of duck plague virus. Naturally duck plague virus can be transmitted directly by contact and indirectly by contact with a contaminated environment. The aquatic environment of ducks provides a common vehicle for feeding, body support, drinking water, and a natural means for viral transmission from infected to susceptible Anseriformes (Leibovitz, 1972). Once an infection has been established, it can be maintained in dense populations such as are present on domestic duck farms without open water. Carrier states have been suspected in the spread of duck plague, but scientific evidence to substantiate this hypothesis has not been reported (Leibovitz, 1972). Egg transmission has not been studied, but the virus has been found in the eggs present in the cloaca of ducks dead of duck plague (Newcomb, 1968).

Clinical Signs

In domestic ducks the incubation period ranges from 3 to 7 days; and early in the course of the disease, sudden death of ducks in good condition may be the only clinical sign (Leibovitz and Hwang, 1968b). Mortality may range from 5% to 100%, and egg production may drop in breeder flocks as much as 25% to 40% (Leibovitz and Hwang, 1968b). As the disease progresses through the flock, photophobia and increased lachrymation causing a circle of wet feathers around the eyes are noted. Later the ocular and nasal discharges become purulent, and the eyelids are pasted together (Dardiri and Gailiunas, 1969). Ducks are anorexic but are polydipsic. The birds are droopy, have ruffled feathers, and a watery or bloody diarrhea which soils the feathers around the vent. Affected ducks are weak, depressed, and often unable to stand. They assume a setting position and creep with wings extended when forced to move (Dardiri and Gailiunas, 1969). If the ducks are forced to enter the water, they float around and struggle back to land. The quack may become hoarse, and in male ducks prolapse of the penis may be seen (Jansen, 1964a; Leibovitz, 1968).

Gross Lesions

Gross lesions of duck plague probably represent maximum tissue destruction. Specific digestive system exanthematous mucosal lesions are found in the esophagus, ceca, colon, and

cloaca. The lumen of the entire digestive tract may be filled with a hemorrhagic exudate. Esophageal mucosa may be covered with a yellowish elongated pseudomembrane which covers hemorrhagic foci. A hemorrhagic ring may be present at the esophageal-proventricular junction (Leibovitz, 1969). The mucosal and serosal surfaces of the intestine are dark red, and circular areas of hemorrhage are present in the annular bands which are visible on the serosal surface (Dardiri and Gailiunas, 1969). Cecal and cloacal content is soft and bloody (Dardiri and Gailiunas, 1969). The cloacal mucosa is dark red and covered by a grey cheesy pseudomembrane; or small hemorrhagic foci covered by a crusty yellow exudate may be present.

The lymphoid tissue is also involved. A small dark spleen with dark foci is present in infected birds. The thymus surface is reddened and speckled with hemorrhages, and edematous fluid may surround the thymus in the posterior cervical region (Leibovitz, 1971). In young ducks the bursa of Fabricius is hyperemic with multiple hemorrhages. Later a diphtheritic membrane and inspissated exudate fill the bursal lumen (Leibovitz, 1971).

Numerous petechial hemorrhages are present on the visceral surface of parenchymatous organs such as the pancreas and kidney. Petechiae and ecchymoses are present on the endocardium and epicardium. Ovarian follicles are hyperemic

and hemorrhagic, and some of the follicles may rupture (Jansen, 1964a). The liver is paler than normal with pinpoint hemorrhages in acute cases. In more chronic cases the liver is darker and often bile stained. Multiple small white foci are often noted in the liver (Leibovitz and Hwang, 1968b).

In duck embryos miliary foci of necrosis are present in the liver 4 days post-inoculation (Gailiunas and Dardiri, 1970). Petechiae are present throughout the embryonal tissues, and plaques are present on the chorioallantoic membranes (Jansen, 1961).

Microscopic Lesions

Microscopic lesions in the gastrointestinal tract are generalized congestion with extravasation, and degeneration and necrosis of the epithelial cells of the mucosa. Portions of the detached necrotic epithelium are invaded by rod-shaped microorganisms (Dardiri and Gailiunas, 1969). Hyperemia, edema, and heterophils are present in the lamina propria. Subserosal hemorrhage and edema are also present. Intranuclear inclusion bodies have been seen in epithelial cells of the esophagus and cloaca. Epithelial cell necrosis in the esophagus and cloaca results in erosions. The mucous glands in the lamina propria of the esophagus undergo coagulative necrosis (Dardiri and Gailiunas, 1969). Leibovitz (1971) describes vascular lesions in the intestine. The vascular

wall is severely altered with the peripheral accumulation of heterophils. Vascular lesions which involve principally the capillaries, venules, and smaller veins have been reported. Intravascular thrombi consisting of cells with inclusion bodies are seen along with extravasation. Vascular walls around emboli are degenerate. Inclusion bodies are present in some endothelial cells (Leibovitz and Hwang, 1968b). Others have been unable to find cells with inclusion bodies in vessels (Dardiri and Gailiunas, 1969). Lesions in the annular bands are described as hemorrhagic infarcts with a free layer of blood present which separates the lymphoid tissue from the mucosa which is undergoing coagulative necrosis and is covered by a pseudomembrane (Leibovitz, 1971). Calcium salts are deposited on some of the degenerated intestinal epithelial cells. In breeder ducks congestive and necrotic alterations may extend from the cloaca up into the oviduct.

Leibovitz (1971) describes the presence of severe and consistent lesions in the reticuloendothelial tissues. Lymphoid follicles are depleted, and karyorrhexis and pyknosis of lymphocytes are described. The cytoplasm of reticular cells subdivides and forms oval pale-staining bodies (Leibovitz, 1971). Intranuclear inclusion bodies are present in the reticuloendothelial cells of the spleen, lymphoid nodules, and thymus. In the thymus coagulative necrosis of

the medullary reticular cells and destruction of the cortical lymphocytes are pronounced (Leibovitz, 1971).

Microscopic hepatic lesions are present in both adult Anseriformes and in duck embryos. Focal areas of necrosis are present in the hepatic parenchyma and tend to localize in periportal areas (Dardiri and Gailiunas, 1969). Intranuclear inclusion bodies are present in the hepatocytes. The inclusion bodies are circular, surrounded by a clear halo, stain purple with hematoxylin and eosin, pink with Giesma, and are Feulgen positive (Dardiri and Gailiunas, 1969). In duck embryos inclusion bodies first appear in the hepatocytes 4 days post-inoculation. Their number increases as hepatic necrosis progresses, and the inclusion bodies are more numerous in hepatocytes around foci of necrosis (Gailiunas and Dardiri, 1970). Focal hemorrhages and foci of necrosis are present in the pancreas.

Immunization

Duck plague virus may be attenuated by serial passage in chicken embryos (Dardiri, 1969). A group of experimental ducks immunized by modified live vaccine was shown to be protected against experimental challenge as soon as 10 hours after vaccination. This protection was ascribed to the interference phenomenon (Jansen, 1964b). In another experiment ducks and geese were vaccinated with modified live vaccine and

challenged 85 days later. Two of 9 ducks died, while 11 of 12 geese succumbed to challenge (Butterfield and Dardiri, 1969b). Pasteurella was isolated from the dead birds; therefore, the results may not be valid. In more recent experiments it has been shown that two inoculations of modified live vaccine gave better protection, although no correlation could be drawn between antibody titers and protection (Toth, 1970, 1971a). Serum antibody titers dropped rapidly in vaccinated birds (Toth, 1971a).

Killed vaccines inoculated intramuscularly have been compared with modified live vaccines given orally. The killed products inoculated intramuscularly were better immunogens than orally administered live vaccines (Butterfield and Dardiri, 1969a). In later experiments live vaccines inoculated intramuscularly were more efficacious than killed vaccines (Toth, 1970). Vaccinated ducks did not shed the vaccine virus (Toth, 1971b).

Effect of Lead on Diseases

Several factors are involved in lead toxicosis of waterfowl. Eating habits of ducks bring them into contact with lead shot used by hunters. Lead shot are mistaken for grit or seed by feeding waterfowl. Shot lodge in the ventriculus where they are slowly ground up, and lead is absorbed from the intestinal tract. Diet also affects lead toxicosis in

waterfowl. Ducks maintained on cracked corn were found to be more susceptible to lead toxicosis than ducks on a well-balanced diet (Locke et al., 1966). Although acid-fast intranuclear bodies form within 24 hours in experimental rats (Choie and Richter, 1972b), the formation of this index of lead toxicosis in ducks was found to be influenced by the number of shot ingested and the length of exposure (Locke et al., 1966).

Clinical signs of lead toxicosis observed in waterfowl are anorexia, weight loss, weakness, drooping wings, inability to fly, ataxia, and greenish diarrhea. At necropsy birds are emaciated and lack body fat. There is serous atrophy of fat in the coronary groove, the gall bladder is enlarged, and the proventriculus is often impacted with food (Hartung, 1971; Trainer and Hunt, 1965a, 1965b). Microscopically many hepatocytes are vacuolated and contain acid-fast intranuclear inclusion bodies. Hemosiderin is present in many hepatocytes and Kupffer cells. Elevated serum glutamic pyruvic transaminase also indicates hepatic damage (Rozman et al., 1974).

Although some lead is excreted by the liver (Klaassen and Shoeman, 1974), lead is primarily excreted by the kidney (Vostal and Heller, 1968; Goyer, 1971). Lead causes cellular degeneration, cloudy swelling, and hyaline droplet degeneration in the renal proximal convoluted tubules; and acid-fast intranuclear inclusion bodies are present in cells of the

proximal convoluted tubules (Locke et al., 1966). Using autoradiographic techniques it was demonstrated that cellular proliferation was increased in these tubules to compensate for cellular degeneration, and even cells with intranuclear inclusion bodies showed evidence of DNA synthesis (Choie and Richter, 1972a).

Lead causes anemia and accumulates in the bone in chronic toxicosis (Buck et al., 1973). Ionic lead has an affinity for erythrocytic membranes, and this interaction causes the erythrocyte to lose potassium and water. These erythrocytes are fragile, and their premature destruction causes the anemia initially noted in lead poisoning. In the bone marrow lead interferes with hemoglobin formation by its inhibitory effect of δ-aminolevulinic acid (ALA) dehydrase, and large amounts of δ-ALA appear in the plasma and urine (Blumenthal et al., 1972; de Bruin, 1971). Lead is also thought to inhibit the incorporation of iron into the heme molecule. Numerous promegaloblastic-like cells with basophilic cytoplasm and fine chromatin network accumulate in the bone marrow. Maturation of these cells is arrested, and they are not released into the peripheral blood (Bates et al., 1968). Basophilic-stippled erythrocytes are present in the peripheral blood, and the size and hemoglobin content of these cells are decreased (Johns, 1933). Under blue-ultraviolet light the abnormal content of

these cells causes them to fluoresce red (Barrett and Karstad, 1971).

Considerable evidence is available that low levels of lead have an adverse effect on the resistance of the body to disease. For example, Gainer (1973) found that low levels of lead administered to mice decreased the resistance of mice to encephalomyocarditis virus, but did not reduce the protective effect of mouse interferon inoculated into mice. Since Newcastle disease virus did not convey the same protection as interferon, it was concluded lead interfered with interferon production in mice. In contrast, Vengris (1973) demonstrated that a low level of lead did not inhibit the production of interferon in chickens. Hemphill et al. (1971) and Hemphill (1973) demonstrated low levels of lead decreased the resistance of mice to Salmonella typhimurium. Properdin levels in these mice were absent or markedly reduced, and antibody production was reduced. Reduced antibody production against sheep erythrocytes has also been reported in mice with subclinical lead toxicosis (Koller and Kovacic, 1974). Whether this reduced production of antibody was due to reduced phagocytosis by the reticuloendothelial system (Trejo et al., 1972) or due to a direct depression of serum globulins was not determined (White and Harvey, 1972).

Experimentally low levels of lead have been found to increase the susceptibility of mice (Selye et al., 1966) and

baboons (Hoffmann et al., 1974) to endotoxins. Inoculation of lead alone in baboons caused vacuolation of Kupffer cells and the accumulation of dense aggregates in phagosomes. A few hepatocytes had degenerative changes. In baboons inoculated with endotoxin alone fibrin deposits were present in the sinusoids, and degenerative changes were evident in a few Kupffer cells and hepatocytes. The inoculation of lead and endotoxin produced marked morphological changes in the baboon liver. There were large deposits of fibrin, cellular detritus, and inflammatory cells in the sinusoids. Kupffer cells and hepatocytes were necrotic; and autophagic vacuoles, multivesicular bodies, and fat vacuoles were present in the cytoplasm (Hoffmann et al., 1974).

Disease in Germfree Animals

Gnotobiotic and germfree animals are isolated in a microecology. In this environment lymphoid tissue is seldom stimulated by antigenic products, and its development is delayed. Spleens of 4- to 8-week-old germfree birds contain fewer lymphocytes around arteries, and germinal centers are greatly reduced in number. This reduced number of lymphocytes causes the Schweigger-Seidel (reticular) sheaths to appear prominent microscopically. Although the size of the thymus (organ weight per unit of body weight) is equal in germfree and conventional chickens, secondary nodules are virtually

absent in the outer portion of the medulla; and plasma cells are difficult to find in germfree chickens. In contrast, secondary nodules and plasma cells are commonly found in the thymus of conventional birds (Thorbecke et al., 1957). Thymic involution occurs at the same age in germfree and conventional chickens (Uno et al., 1968). Involution of the bursa of Fabricius of germfree and conventional chickens also occurs simultaneously. Follicles of the bursa of Fabricius in both groups of birds are structurally similar, but more plasma cells are present in the interfollicular connective tissue of conventional chickens than in germfree birds.

Serum globulin levels also indicate the lymphoid tissue of germfree animals has not developed and produced antibodies. Gamma globulin levels in germfree birds are greatly reduced at 8 weeks of age. Serum albumin levels in contrast are slightly elevated; while β globulin levels are slightly decreased (Thorbecke et al., 1957). After antigenic stimulation germfree animals produce IgM followed by IgG antibodies as previously demonstrated in conventional animals (Kim et al., 1968; Watson et al., 1968).

In one study germfree animals were found to have a slightly but consistently greater immune competence than their conventional counterparts, which might explain why few neoplasms were found in germfree animals (Walburg, 1973). Germfree mice were also found to produce more interferon when

inoculated with Newcastle disease or vaccina viruses than conventional controls (Starr and Considine, 1968).

Histologic differences also were present in the adrenal gland, bone marrow, lung, and liver. Both the zona glomerulosa and fasciculata of the adrenals of germfree rats were poorly developed when compared with conventional controls. A decrease in number and size of cytoplasmic lipid droplets, differences in intramitochondrial structures, decrease in size and amount of smooth endoplasmic reticulum, and an increase in the number of dense cytoplasmic granules all suggest that the function of the adrenal glands in germfree animals was decreased (Wakabayashi et al., 1968). Hematopoietic activity was decreased in the femur, tibia, humerus, radius, third and fourth lumbar vertebrae, and tail vertebrae of germfree rats. Numbers of granulocytic cells in the marrow were markedly reduced, and this was reflected by a decreased number of granulocytes in the peripheral blood (Hashimoto and Hashimoto, 1968). Despite the decreased number of circulating granulocytes and the absence of serum antibodies, the reticuloendothelial system of germfree animals had an innate ability to remove antigens from the blood, although antibody was found to accelerate this removal (Watson et al., 1968). Alveolar walls of germfree guinea pigs contained fewer cells than controls, and the epithelium lining the bronchioles was flat with a thin basement membrane beneath. Lymphoid nodules were absent in

the lung of germfree guinea pigs, and argentaffin fibers in alveolar septa and bronchiolar walls were delicate and poorly branched when compared with controls (Tanami et al., 1968). The number of lymphocytes around the hepatic portal triads was decreased in the germfree chickens (Thorbecke et al., 1957). Decreased levels of alanine aminotransferase and glucose-6-phosphate dehydrogenase also were detected in the livers of germfree rats (Kotake et al., 1968). Bile acid oxidation, catabolism, and the turnover of bile acids proceeded at a slower rate in germfree chickens and rats; and total bile acid output was also decreased (Eyssen et al., 1968).

Several differences have been reported in the digestive tracts of germfree animals. Conventional chickens older than 2 weeks contained well-developed lymphoid tissue with germinal centers, blast cells and plasma cells in the ileocecocolic junction and ceca. Four-week-old germfree counterparts did not contain any secondary nodules or plasma cells in these organs, although irregularly shaped accumulations of small lymphocytes were frequently found. In 6- to 8-week-old germfree birds an occasional secondary nodule was found along with a few plasma cells (Thorbecke et al., 1957). Ceca of germfree rodents were enlarged and contained large amounts of fluid. This dilation may be caused by an elevated level of fecal kallikrein (Wiseman and Cole, 1968). Colonic temperature of germfree rats was reported to be about 1° C lower in germfree

rats than in conventional rats (Levenson et al., 1968). In germfree animals intestinal villous epithelium was replaced at a slower rate. This longevity decreased the rate of protein synthesis (Gordon, 1968) and increased the level of disaccharidases in the intestinal epithelium (Reddy and Wostmann, 1968).

There were no morphologic or histologic differences in the response of germfree and conventional mice to acute oral injury except for superficial microbial colonization that occurred in conventional mice. Vascular changes, sequential appearance of inflammatory cells and re-epithelialization were complete by the ninth day. In contrast, reaction to chronic injury was different in germfree and conventional mice. Two weeks after a silk ligature was placed at the cervical margin of the first molar, an inflammatory response consisting of neutrophils and lymphocytes was present in the gingiva of conventional mice, but not in the gingiva of germfree mice (Rovin, 1968).

Germfree animals have been used to study the pathogenesis of several diseases, and some interesting results have been obtained. Some pathogens do not produce disease in germfree animals. For example, Entamoeba histolytica in germfree guinea pigs (Phillips, 1968) or Histomonas meleagridis in gnotobiotic turkeys (Bradley and Reid, 1966) did not produce a disease in the absence of intestinal flora. The presence of

one or more bacteria was required for these protozoa to invade the intestinal wall. Another protozoan, Eimeria tenella, invaded the intestinal tract; but its pathogenicity was greatly reduced. In this instance the reduced number of macrophages in the intestinal submucosa of germfree chickens to transport sporozoites to gland fundi was probably responsible for the reduced pathogenicity (Johnson et al., 1973). In yet another protozoan infection, E. maxima, which does not use macrophages in its life cycle, the diseases produced in germfree and conventional chickens were similar (Rice et al., 1973).

Germfree animals have been used in the study of viral diseases to eliminate the effect of secondary infection and to obtain uniformly susceptible animals. These experiments have produced some unexpected results. For example, canine distemper in gnotobiotic dogs was an extremely mild disease and would not have been detected if temperature records had not been kept. Neutropenia and anemia as seen in conventional animals were not present in germfree dogs. In contrast to conventional dogs, thymic atrophy occurred; and inclusion bodies were difficult to find microscopically in germfree dogs. Microscopically lymphoid depletion was evident, and lymphoid tissue was thought to be the primary site of viral multiplication in both groups of dogs (Gibson et al., 1965). Gross and microscopic lesions of canine hepatitis were similar in germfree and conventional dogs. However, germfree dogs had

a more uniform response to canine hepatitis virus than conventional dogs. Germfree dogs inoculated with the same dilution of canine hepatitis virus had identical fever curves, and the time of death could be predicted within a few hours (Griesemer, 1968).

Germfree cats have been used to study the pathogenesis of feline infectious peritonitis, feline infectious enteritis, feline picornavirus infection, and feline viral rhinotracheitis. Experimental inoculation of germfree and conventional cats with feline infectious peritonitis virus produced an identical disease syndrome. Clinical signs observed in experimental cats were ascites, weight loss, partial anorexia, and persistent fever. Three out of nine inoculated cats died during the second and third weeks post-inoculation. Lesions in experimental animals were restricted to the abdominal cavity and consisted of an excess abdominal fluid and fibrinous exudate on the serosal surface of abdominal viscera (Wolf and Griesemer, 1966). The focal granulomatous inflammation affecting the kidneys, visceral lymph nodes, lung, liver, eyes, and leptomeninges found in natural infectious peritonitis in cats necropsied by Montali and Strandberg (1972) was not reported by Wolf and Griesemer (1966) in experimentally inoculated conventional or germfree cats.

Feline infectious enteritis was not a fulminating disease in germfree cats, and cats had begun to recover by 7 days

post-inoculation. Severe leukopenia was due to absolute lymphopenia followed a few days later by absolute neutropenia. Severe thymic atrophy was due to depletion of lymphocytes which may be the target cells for the virus. Reticuloendothelial cells in the medulla were hyperplastic and contained lymphocytic detritus. Thymic lesions had not been previously reported, but were similar to lesions experimentally produced in specific pathogen-free cats. Although lymphoid tissue in Peyer's patches, mesenteric and mandibular lymph nodes, tonsils, and spleen was well developed, lymphoid depletion, occasionally focal necrosis of lymphoid tissue, and reticuloendothelial hyperplasia were noted microscopically. Nuclei of these hyperplastic cells contained greatly enlarged nucleoli. In the bone marrow of infected germfree cats the myeloid-erythroid ratio was lower than 3.3 previously reported for cats recovering from panleukopenia. Microscopic lesions were not seen in the small intestinal mucosa of germfree cats (Rohovsky and Griesemer, 1967). It has been postulated that the proliferation of crypt cells in germfree cats is so slow that they are resistant to feline infectious enteritis virus (Kent and Moon, 1973).

Clinical signs of feline viral rhinotracheitis in germfree cats consisted of epiphora, odynophagia, paroxysmal sneezing, coughing, anorexia, dyspnea, and listlessness and were similar clinically to the disease in conventional cats.

Initially the nasal discharge was serous but became viscid and opaque on the fifth day post-inoculation. Two of eight inoculated germfree cats died. In germfree cats the virus rapidly destroyed virtually all of the nasal epithelium. A neutrophilic infiltration in the submucosa was apparently elicited by products of epithelial necrosis. Osteolytic lesions in the turbinate bones may have been due to (1) severe necrotizing rhinitis, (2) ischemic necrosis caused by vascular lesions, or (3) viral infection of osteogenic cells. Regeneration of nasal epithelium correlated with the appearance of lymphocytes in the nasal submucosa. The tracheal lesions of conventional and germfree cats indicated the pathogenicity of feline rhinotracheitis virus was not dependent upon the synergistic activity of respiratory microbial flora (Hoover et al., 1970).

Uniformly susceptible animals were needed to study some viral diseases. For example, inconclusive results had been obtained when conventional pigs were inoculated with porcine enteroviruses. The nonsuppurative polioencephalitis produced in germfree pigs was similar to that observed sporadically in conventional pigs, but consistent results obtained in the germfree pigs enabled the investigators to reach definitive conclusions about the pathogenicity of the porcine enteroviruses (Holman et al., 1966; Long et al., 1966).

Germfree animals were also needed to determine the virulence of feline picornaviruses. After exposure of

germfree cats to feline picornavirus-255, depression, anorexia, and pyrexia were observed; and ulcers were present on the lingual or palatine mucosa in 8 of 12 cats. The initial gross pulmonary lesions were multiple bright red areas of consolidation scattered throughout all lobes of the lung. Microscopically the first pulmonary changes were congestion, infiltration of neutrophils into alveolar walls, and pyknosis and hypertrophy of pneumocytes and mesothelial cells. By 4 days post-inoculation large macrophages were in the alveoli. The predominant lesion observed 7 to 14 days post-inoculation was an interstitial pneumonia characterized by adenomatoid proliferation of large, round, type II alveolar pneumocytes and predominantly macrophages in the alveoli. From 2 to 3 weeks post-inoculation a resolving pneumonia was noted microscopically. The earliest histologic change in the oral mucosa was pyknosis and cytoplasmic eosinophilia of small clusters of cells in the nonkeratinized stratum corneum or stratum spinosum. Separation and retraction of cell membranes and neutrophil infiltration followed. Keratinocyte necrosis spread from the initial foci and formed an ulcer. Lesions observed in germfree cats were similar to those in previous cases of feline pneumonia in conventional cats (Hoover and Kahn, 1973).

LABORATORY TECHNIQUES

Source and Preparation of Virus

Duck plague virus (DPV) used in this study was isolated from the liver of a mallard duck which died in the epidemic at Lake Andes, South Dakota, in January and February, 1973. Five grams of liver and 20 ml of trihydrochloride buffered tryptose broth, containing 5,000 units of penicillin, 1 μg streptomycin, 50 μg Fungizone, and 50 μg kanamycin sulfate/ml, were placed in a blender and chopped for 1 minute at 25,000 rpm. After centrifugation for 15 minutes at 1,600 x g, one ml of supernate was placed in each Leighton tube containing primary duck embryo fibroblasts. Virus was allowed to adsorb for 1 hour before the inoculum was removed and replaced by fresh medium, Earle's medium with 1% amino acid and 2% vitamins added. Third passage tissue culture virus was harvested and used for duck inoculation. Virus titer was determined by inoculation of 12-day-old duck embryos (Erickson et al., 1974) and the median lethal dose calculated using Karber's technique (Lennette, 1969). The viral titer of the solution used to inoculate ducks was 10^4 duck embryo median lethal doses ($DELD_{50}$) per ml.

Pathogenesis Study

Trial I

Forty white Pekin ducks were obtained from a commercial source, placed in an isolation room with a temperature of 70° F and a relative humidity of 45%, and fed a routine ration until they were 6 weeks old. At 6 weeks of age, 15 of the ducks were placed in another room; and each of the 25 remaining ducks was given 1 ml of duck plague virus containing 10^4 $DELD_{50}$. Three infected and two control ducks were killed daily for the next 5 days. Necropsies were conducted on all birds that died or that were killed during the 5-day period. Tissue samples were placed in 10% neutral buffered formalin. Two sections of esophagus (one near the anterior end and the other at the esophageal-proventricular junction), a section of proventriculus, ventriculus, liver, kidney, spleen, adrenal gland, bursa of Fabricius, heart, lung, trachea, the entire brain, split femur, cloaca, and annular bands were collected. Sections of duodenum, middle jejunum, jejuno-ileal junction containing Meckel's diverticulum, terminal ileum, ceca, and colon were placed in individual containers of formalin to facilitate identification. Adjacent portions of the above specimens were frozen in liquid nitrogen and stored in a Revco[1] freezer (-60° C).

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[1]Revco, Inc., Deerfield, Michigan.

Trial II

The above experiment was repeated using another 40 ducks. Small samples, 1 mm thick, were quickly removed from the esophagus, small intestine, cloaca, liver, spleen, bursa of Fabricius, and thymus of each duck necropsied. Each sample was placed in 2.5% glutaraldehyde buffered to pH 7.4 with sodium cacodylate buffer.

Trial III

To obtain a more uniform sequence of lesions in the liver, 0.5 ml DPV containing 10^4 $DELD_{50}$ per ml was inoculated intravenously via the jugular vein of thirty 6-week-old white Pekin ducks. Ten controls were kept in a separate room. One control and two inoculated ducks were randomly selected and killed at 12-hour intervals for 72 hours. One mm thick sections of liver were quickly removed from each duck and placed in 2.5% glutaraldehyde buffered with sodium cacodylate buffer to pH 7.4.

Effect of Lead on Duck Plague

Trial IV

Forty 2-week-old ducks were used in this experiment. Each duck was randomly assigned to one of four groups of 10 ducks. Three ml of blood were taken from the jugular vein and placed in heparinized Vacutainer[1] tubes. Packed cell

[1]Vacutainer B-D, Becton, Dickinson and Company, Columbus, Nebraska.

volumes and preinoculation blood lead levels were determined on these samples. Each duck was also weighed. Ducks in group number 1 were wing banded with numbers 1 through 10. Each duck was given 16 number 6 lead shot orally with a stomach tube long enough to reach the proventriculus. Ducks in group number 2 (wing bands 11-20) were given 8 number 6 lead shot orally, and ducks in group number 3 (wing bands 21-30) were given 4 number 6 lead shot orally. The first three groups of ducks were placed in one room. Group number 4 ducks (wing bands 31-40) were used as controls and were placed in another room to prevent them from ingesting lead shot which occasionally pass through the gastrointestinal tracts of ducks.

Once a week for 4 weeks after giving lead orally, 5 ml of blood were taken from the jugular vein of each of the 40 ducks and placed in heparinized Vacutainer tubes. Packed cell volume and blood lead level were determined on each sample. At the end of the 4 weeks, each duck was given orally 0.5 ml of DPV containing 10^4 $DELD_{50}$ per ml. Birds were observed several times daily. Shortly after death birds were necropsied. Lead shot found in the ventriculus were counted. Samples of liver, kidney, and femur were frozen for future lead analysis. Sections of liver, kidney, femur, spleen, lung, brain, thymus, bursa of Fabricius, and intestine were placed in 10% formalin for histologic examination.

Trial V

Forty 2-week-old ducks were used in this experiment. Each duck was randomly assigned to one of five groups. Three ml of blood were taken from the jugular vein of each duck and placed in heparinized Vacutainer tubes. Ducks with wing band numbers 64, 65, 71, 73, 78, 86, 89, and 96 were in group number 1 and were given 4 number 6 lead shot orally with a stomach tube. Those with wing band numbers 61, 66, 81, 82, 88, 91, 95, and 97 were in group number 2 and were given 8 number 6 lead shot. Ducks in group number 3 with wing band numbers 60, 70, 74, 77, 80, 85, 93, and 99 were given 16 number 6 lead shot. Ducks in group number 4 with wing band numbers 63, 68, 69, 76, 79, 84, 92, and 98 were a control group which were removed and placed in a separate room. Ducks with wing band numbers 62, 67, 72, 75, 83, 87, 90, and 94 were left in the same room with groups 1, 2, and 3. Four weeks after lead shot was given orally, 5 ml of blood were collected from the jugular vein of each duck and placed in heparinized Vacutainer tubes. Each duck was then inoculated orally with 1 ml of DPV containing 10^2 $DELD_{50}$ per ml. This dilution of virus contained 40 plaque-forming units per ml when placed on primary duck embryo fibroblast. Ducks were observed closely, and a necropsy was conducted on each duck that died. Lead shot found in the ventriculus were counted. Samples of liver, kidney, and femur were frozen for lead analysis. Sections of

liver, kidney, femur, spleen, lung, brain, thymus, bursa of Fabricius, and intestine were placed in 10% formalin for histologic examination.

Gnotobiotic Ducks

Trial VI

Seven 4-week-old gnotobiotic ducks were obtained in an isolator from Animal Supply at the National Animal Disease Center[1] (Luckey, 1963) and placed in an isolation room. Using aerobic and anaerobic bacterial culture techniques, Animal Supply found the isolator contained two bacteria, Escherichia coli and Streptococcus faecalis. Twelve 4-week-old conventional ducks hatched at the same time were delivered and placed in a separate isolation room. One duck was removed from the isolator by placing it in the small air chamber. The inner portion was covered, and the covering over the outer portion of the air lock was removed. An autoclaved sealed plastic container containing hypodermic needles, syringes, and a 10 ml sealed vaccine bottle containing 10 ml DPV was placed in the air lock. The air lock was then sprayed with a 2% peracetic acid solution containing 1% detergent. The outer covering was placed back on the air lock and sealed. Plugs were removed from the outer seal, and the air lock was filled with the above peracetic acid spray; and then the plugs were replaced and sealed. Forty-five minutes later the inner

[1]National Animal Disease Center, Ames, Iowa 50010.

entrance was opened; and the virus, syringes, and needles were put into the isolator. After closing the air lock, each duck was given 1 ml of viral suspension orally. The 11 conventional ducks were also inoculated orally with 1 ml of the same virus. Ducks were observed closely.

Necropsies were conducted on the uninfected gnotobiotic ducks removed from the isolator, one uninfected conventional duck, and on all inoculated ducks that died. Tissues were fixed in 10% formalin as described for conventional ducks.

Preparation of Tissues for Light Microscopy

Selected portions of each formalin-fixed specimen were placed in tissue capsules, dehydrated in graded alcohols, cleared with a clearing agent,[1] and embedded in paraffin using an autotechnicon.[1] Sections 6 μm thick were prepared, and routine hematoxylin and eosin (H & E) stains were used on all sections (Luna, 1968).

Preparation of Tissues for Electron Microscopy

Tissues were fixed for 2 hours in glutaraldehyde, washed twice for 30 minutes in sodium cacodylate buffer, post fixed in 2% osmium tetroxide, diluted 1:1 with sodium cacodylate buffer for 2 hours, washed again twice for 30 minutes each in sodium cacodylate buffer pH 7.4, dehydrated in graded alcohols, placed in propylene oxide twice for 20 minutes, placed

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[1]Technicon Corporation, Tarrytown, New York 10591.

in a 1:1 mixture of propylene oxide and Epon 812[1] for 2 hours, put in capsules containing Epon and polymerized in a 60° C oven for 24 hours (Luft, 1961). Ultrathin sections were cut with an LKB 4800A Ultratome[2] using a diamond knife. Sections were placed on a 200 mesh/square inch copper grid, hydrated through graded alcohols, placed in water, stained for 2 minutes in lead citrate, washed in alcohol, dehydrated in graded methyl alcohol, and stained for 5 minutes with uranyl acetate. They were then washed by going back through the graded alcohol to water (Venable and Coggeshall, 1965). Sections were examined with a Philips 200 electron microscope.[3]

Fluorescent Antibody Staining

Gamma globulin from ewes hyperimmunized with duck plague virus, Holland attenuated strain, and conjugated with fluorescein isothiocyanate (FGG) was obtained from Dr. G. A. Erickson.[4] Some gamma globulin (GG) from the same ewes was also obtained (Erickson et al., 1974). Two frozen sections 10 μm thick were cut from each tissue specimen on a cryostat and placed on glass slides. Mounted tissue sections were

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[1]Fisher Scientific Co., Fair Lawn, New Jersey.

[2]LKB-Produkter AB, Stockholm, Sweden.

[3]Philips Electronic Instruments, Mount Vernon, New York 10550.

[4]National Animal Disease Center, Ames, Iowa 50010.

fixed for 10 minutes in acetone at room temperature. After fixation, one of the two slides was flooded with FGG; and this section was designated FASTS. Another was flooded with a 1:1 mixture of FGG and GG. These sections were then incubated at 37° C for 30 minutes in a moist chamber. The globulin preparations were poured off, and the tissue was washed for 10 minutes in phosphate buffered saline and rinsed in distilled water. Three drops of glycerin were placed on the section, and a coverglass was gently placed over it. Tissue sections were examined with a Leitz Ortholux fluorescent microscope[1] (Henry and McDaniel, 1971). Tissues from uninfected animals served as one control, and stained tissues from infected birds with a mixture of FGG and GG served as another control.

Lead Analysis

Bone

All of the muscular tissue was removed from the femurs prior to preparation for lead analysis. Bones were dried by placing them in a 110° C oven for 2 hours. Each bone was weighed and placed in the oven for another hour. This hourly process was repeated until a constant weight was obtained. Samples were weighed, crushed with a mortar and pestle, and 1 ml of 20 ppm magnesium sulfate, aqueous solution, was added per gram of tissue. They were placed in a muffle oven at

[1]Leitz, Inc., New York, New York.

250° C for 2 hours. The temperature was then increased to 550° C and held at this point overnight. After cooling, the ash was moistened with 10 ml of distilled water and transferred to a 100 ml volumetric flask containing 50 ml of 6 N hydrochloric acid. Distilled water was added to fill the volumetric flask, and this solution was analyzed for its lead content using a Perkin-Elmer model 303 atomic absorption spectrophotometer[1] at a wave length of 2,833.1 A. Standard solutions were prepared in 3 N hydrochloric acid containing 0.5, 1.0, 3.0, 5.0, 10.0, and 20.0 ppm lead using lead acetate. Standard solutions were also analyzed by atomic absorption, and readings obtained were plotted on graph paper.

Liver and kidney

Liver and kidney were digested for atomic absorption spectrophotometric analysis. Five grams of tissue were minced into a 125 ml Erlenmeyer flask and covered with concentrated nitric acid. One and one-half ml of concentrated sulfuric acid and 12 to 16 glass beads were added to each flask. Samples were then placed on a steam bath until the tissue was dissolved and the supernate was clear. Digestion was continued over an open flame, periodically adding nitric acid to prevent charring, until a clear yellowish-white liquid remained after nitric acid was driven off and white fumes of

[1]Perkin-Elmer Co., Norwalk, Connecticut 06856.

sulfur dioxide appeared. After cooling, 10 ml of distilled water were added and evaporated over an open flame. Heating was again continued until sulfur dioxide fumes appeared. Contents of the flask were then transferred to a 50 ml volumetric flask, rinsing the Erlenmeyer flask with distilled water. The samples were diluted to volume with distilled water and were then ready for lead analysis using the atomic absorption spectrophotometer. Standard solutions were prepared in 3% sulfuric acid solution containing 0.3, 0.5, 1.0, and 3.0 ppm lead using lead acetate. These solutions were also analyzed by atomic absorption spectrophotometry, and results were plotted on graph paper.

Blood

Five ml of heparinized blood were placed in 16 by 130 mm test tubes. Since samples collected from ducks prior to oral inoculation of shot were only 3 ml, two samples were randomly pooled and analyzed as one sample. Rapid hemolysis was obtained by adding 1 ml of 5% Trition X-100[1] solution and mixing the solution on a vortex mixer. One ml of a 2% 1-pyrrolidinecarbodithioic acid ammonium salt in distilled water was added to each tube and mixed on a vortex mixer. Five ml of water-saturated methyl isobutylketone was then added and shaken vigorously about 60 times. After centrifugation for 10

[1]Rohm and Haas, Perkin-Elmer Co., Norwalk, Connecticut.

minutes at 8,100 x g, the supernate was aspirated and placed in a clean test tube for analysis by atomic absorption spectrophotometry. Standard solutions were prepared by taking 5 ml of duck blood and adding lead acetate to obtain blood samples containing 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, and 5.0 ppm of lead. These samples were then analyzed by the above technique used for unknown blood samples. Readouts from the atomic absorption spectrophotometer were used to make a graph with atomic absorption spectrophotometric data on the horizontal axis and ppm lead on the vertical axis.

RESULTS

Trial I

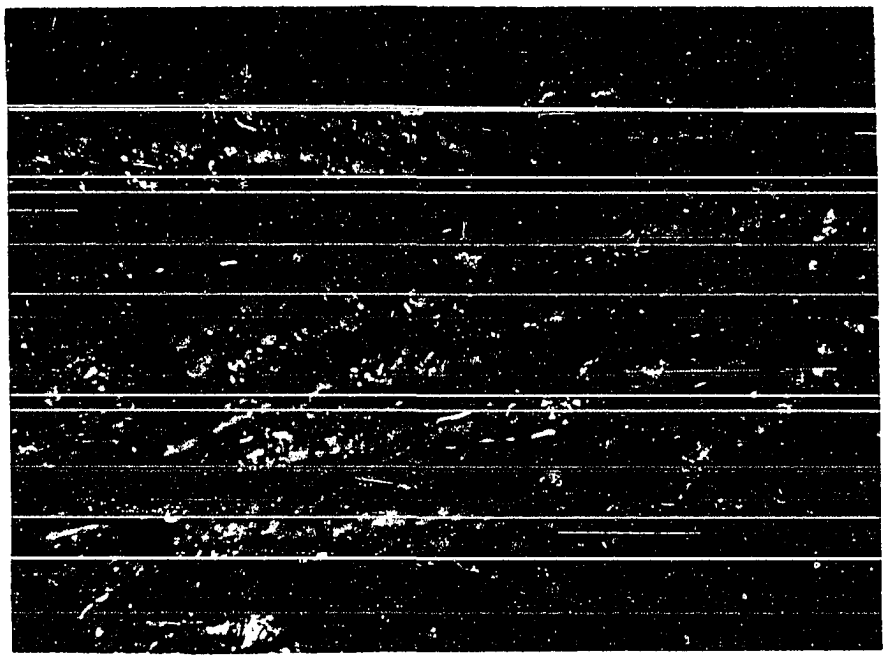
Macroscopic lesions

Esophagus Gross lesions of the esophagus appeared on the third day post-inoculation (DPI). Small white plaques of loosely adhered fibrin were present at the esophageal-proventricular junction, and large amounts of mucus were present in the esophagus. On the fourth and fifth DPI, elevated, tenacious, necrotic yellowish plaques covered submucosal hemorrhages throughout the esophagus in 4 of 13 ducks and at the esophageal-proventricular junction of most ducks (Figs. 1 and 2). Hemorrhagic ulcers were present when plaques were removed.

Large and small intestines A catarrhal enteritis was seen in one duck on the third DPI. On the fourth and fifth DPI, submucosal petechiae, diffuse and focal hemorrhage in annular bands, and a hemorrhagic Meckel's diverticulum were visible from the serosal surface. When the intestine was opened, a diffuse catarrhal enteritis with scattered submucosal petechiae was noted. Annular bands were covered by yellowish diphtheritic membranes. Removal of the mucus, diphtheritic plaques over submucosal petechiae, and diphtheritic membranes on annular bands exposed hemorrhagic ulcers (Figs. 3 and 4). A yellow, tenacious cast or plug was present in the lumen of Meckel's diverticulum. Lesions in the large

Figure 1. Esophagus 4 DPI, Trial I. Multiple small diphtheritic plaques are present on the esophageal mucosa

Figure 2. Esophageal-proventricular junction 4 DPI, Trial I. Small diphtheritic plaques are present at the esophageal-proventricular junction. A large amount of mucus is present on the proventricular mucosa



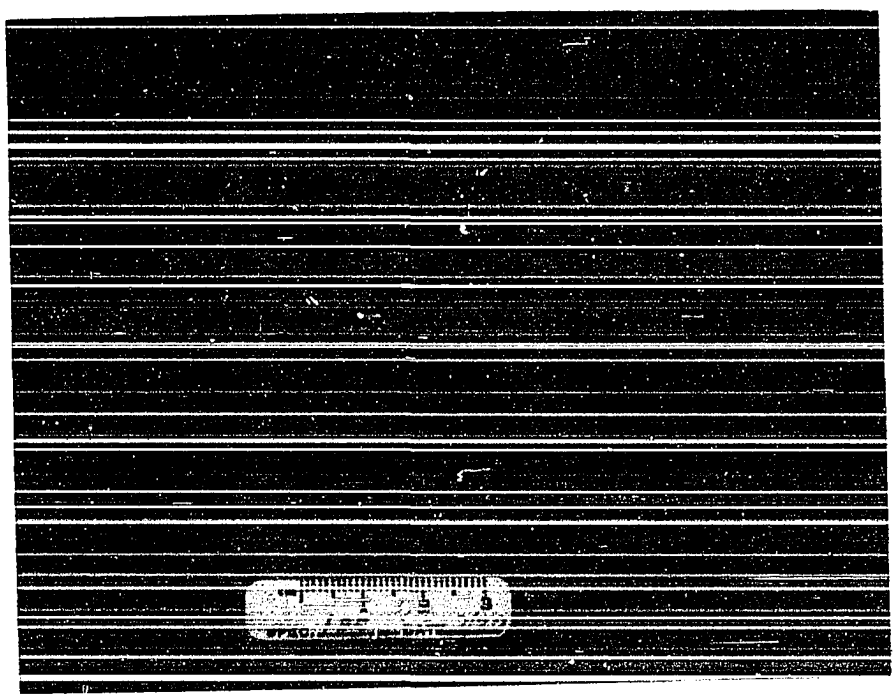
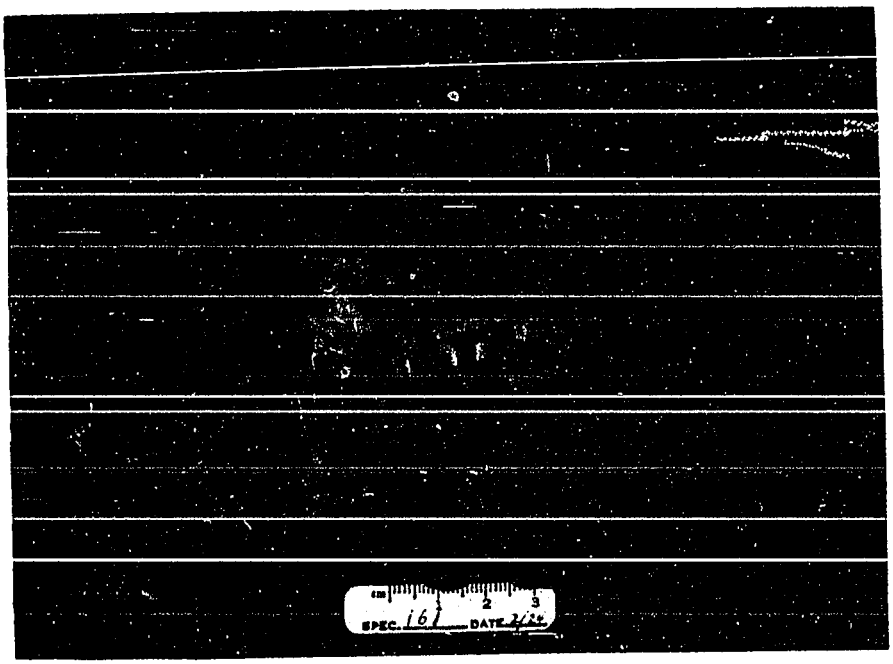


Figure 3. Small intestine 4 DPI, Trial I. A diffuse catarrhal enteritis is present in the four open sections of small intestine. The four hemorrhagic annular bands are covered by diphtheritic membranes. Scattered submucosal petechiae are also present. Meckel's diverticulum on the unopened section of intestine is hemorrhagic

Figure 4. Small intestine 5 DPI, Trial I. Brownish diphtheritic membranes are present on the annular bands in upper and lower sections of small intestine. The membrane has been removed from the second section from the bottom, and a hemorrhagic ulcer is present. Meckel's diverticulum attached to the other section of intestine is hemorrhagic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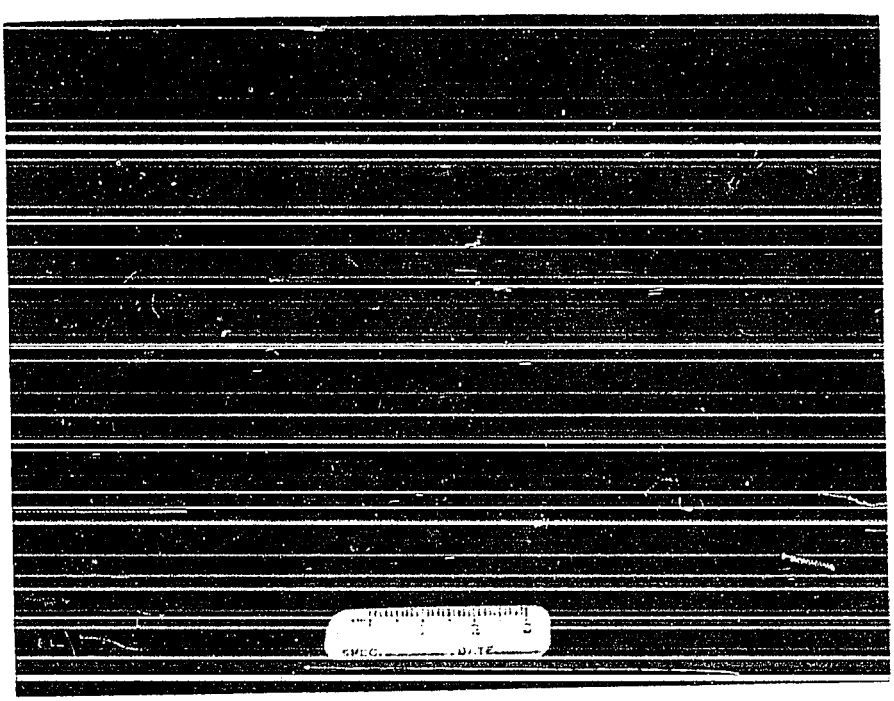


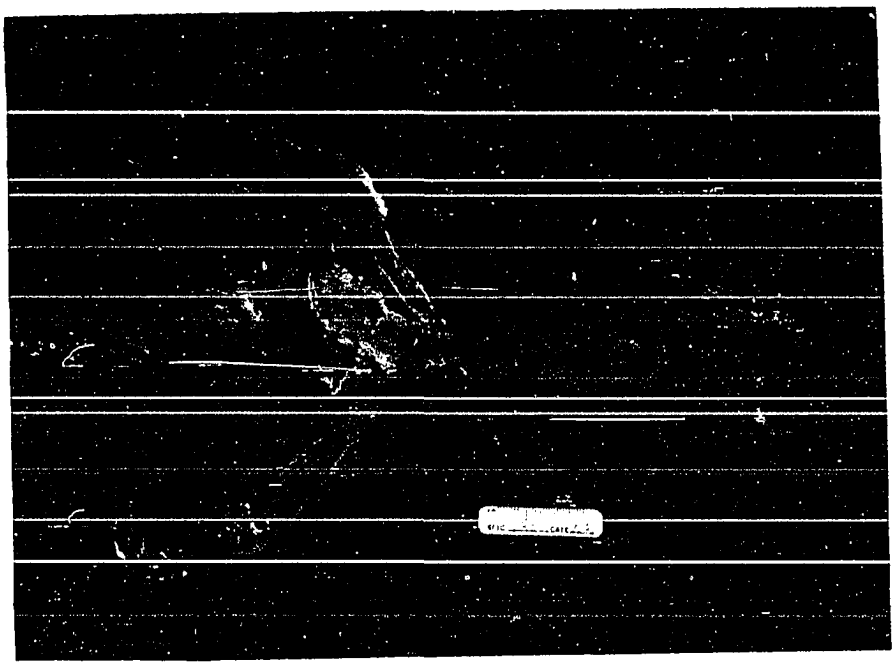
intestine ranged from a catarrhal colitis and typhlitis with scattered submucosal petechiae to an acute fibrinohemorrhagic colitis and typhlitis on the fourth and fifth DPI (Figs. 5 and 6). Removal of the tenacious fibrinous exudate exposed hemorrhagic ulcers.

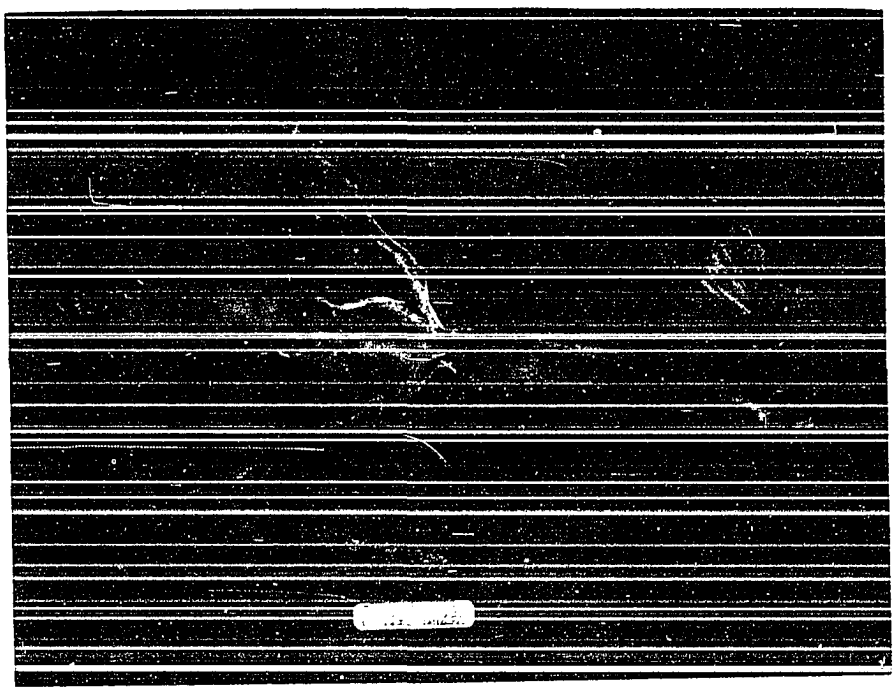
Catarrhal cloacitis with scattered petechiae was noted on the fourth DPI. On the fifth DPI a diffuse to multiple focal hemorrhagic diphtheritic membranous cloacitis was found in 6 of 13 ducks necropsied, while in the other 7 ducks a catarrhal cloacitis with multiple petechiae and ecchymoses was observed.

Thymus, spleen, and bursa of Fabricius Macroscopic lesions were present in all three major lymphoid organs of the duck. The spleen of one duck necropsied 3 DPI had lost its normal mottled appearance and was dark blue. Spleens of ducks necropsied 4 DPI were friable, normal size, and dark red. Diffuse congestion was evident on the cut surface. Spleens in the ducks found dead 5 DPI were dark red and friable. In 2 of 13 spleens white foci of necrosis were visible on the capsular and cut surfaces (Fig 7). Lesions in the bursa of Fabricius were seen on the fourth DPI. Straw-colored gelatinous fluid was present in the peribursal connective tissue of the three ducks necropsied. On the fifth DPI, bursal petechiae were observed in 4 of 13 ducks necropsied. Scattered petechiae and straw-colored gelatinous fluid were found in the thymus of one

Figure 5. Large intestine 4 DPI, Trial I. The posterior ileal annular band between the tips of the ceca is hemorrhagic. Multiple small petechiae are present in the ceca, and diphtheritic membrane covers the anterior part of the cloaca

Figure 6. Large intestine 5 DPI, Trial I. The posterior ileal annular band is hemorrhagic and is located between the tips of the ceca. Hemorrhage and tags of fibrin are present in the ceca. A dark diphtheritic membrane is present in the cloaca, and hemorrhages are present in the cloaca and bursa of Fabricius





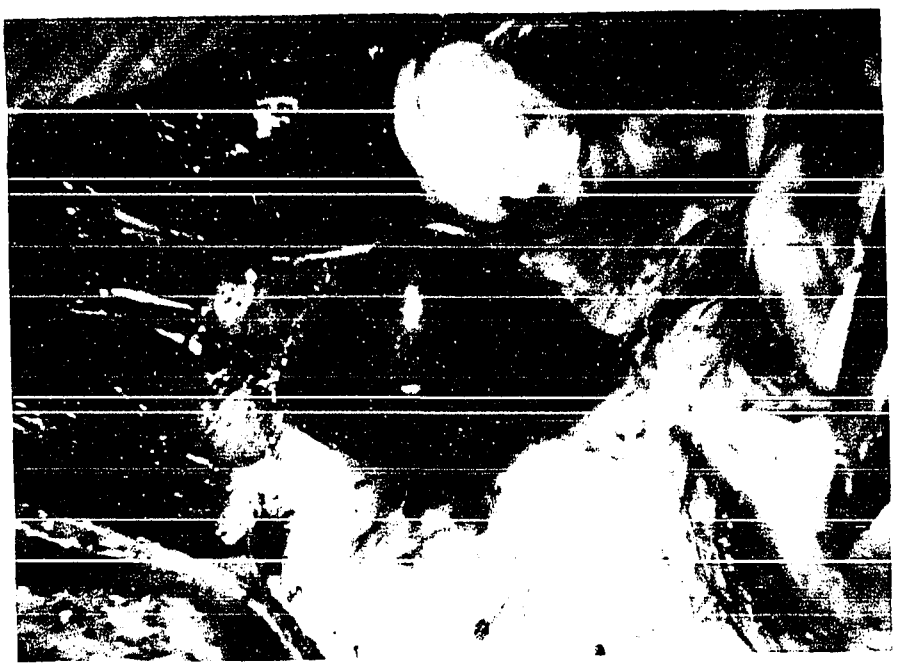
duck necropsied the fourth DPI. Thymic petechiae and perilobular edema were present in 8 of 13 ducks necropsied the fifth DPI.

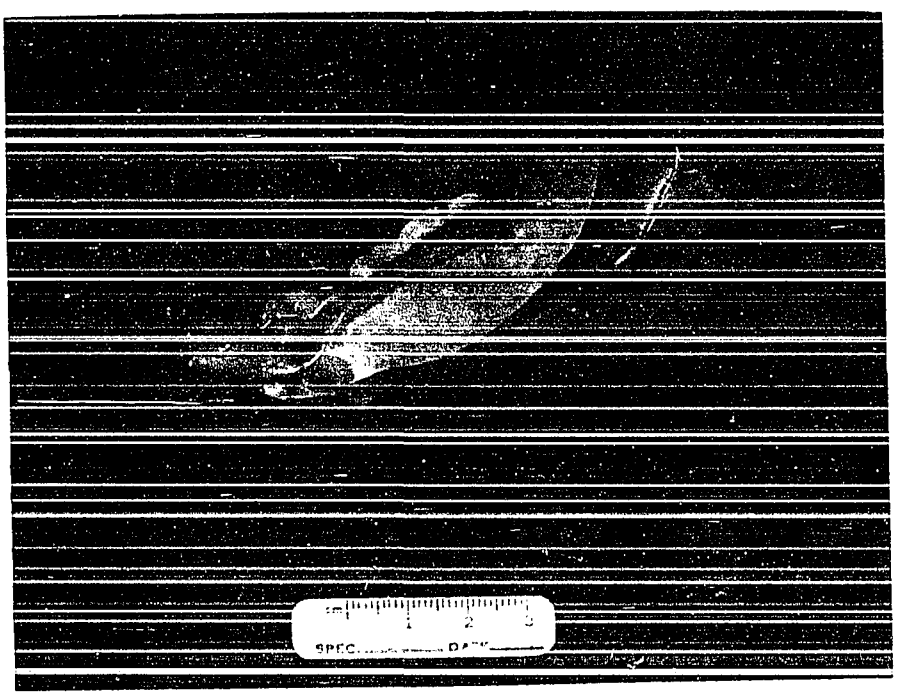
Pancreas Lesions were observed in the pancreases of two ducks necropsied. Scattered petechiae and pinpoint foci of necrosis were present in 2 of 13 pancreases examined the fifth DPI (Fig. 8).

Liver Hepatic lesions appeared on the third DPI. Livers in two ducks were swollen and congested, and the edges of the cut surface bulged. In another duck necropsied the same day, petechiae were also noted beneath Glisson's capsule. On the fourth DPI livers of two ducks were congested and friable with scattered pinpoint (1 mm in diameter) white foci of necrosis and petechiae beneath the capsule. On cross-section focal necrosis and hemorrhages were present throughout the parenchyma, and blood oozed from the surface. In one duck coalescence of subcapsular hemorrhages gave the surface of the liver a "paint brush" appearance. Lesions seen in the livers of the 13 ducks necropsied on the fifth DPI also contained "paint brush" hemorrhages, and white foci of necrosis were visible in 5 of the 13 livers (Fig. 9). In two livers large subcapsular ecchymoses (1 to 2 cm in diameter) were present beneath Glisson's capsule. When these large hemorrhages were

Figure 7. Spleen 5 DPI, Trial I. Multiple small white foci of necrosis are present in the spleen

Figure 8. Pancreas 5 DPI, Trial I. Scattered small white foci of necrosis are visible in the pancreas

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present along the edge of the liver, they extended throughout the parenchyma (Fig. 10).

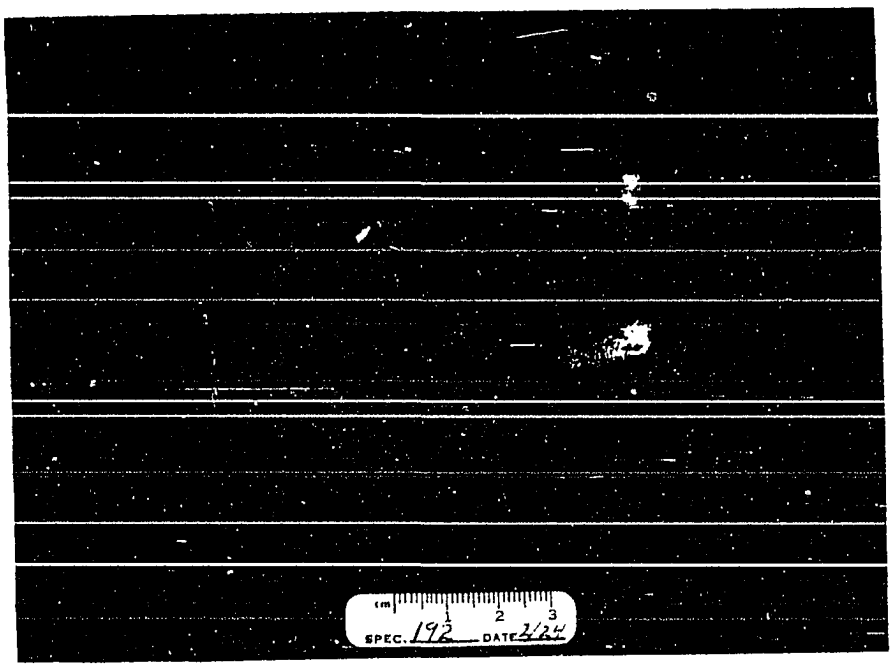
Other organs Petechiae were present around the coronary groove of one duck necropsied 4 DPI. Four of 13 ducks necropsied the next day had petechiae beneath the epicardium and endocardium. Scattered petechiae were observed beneath the pleura in 4 of 13 ducks necropsied 5 DPI. Three of these ducks also had petechiae in the adrenal gland. An occasional petechia was found in the kidney and gonads. The femoral bone marrow in 8 of 13 ducks necropsied 5 DPI was semifluid and dark red.

Microscopic lesions

Esophagus Microscopic lesions developed in the submucosal glands, stratified squamous epithelium, and submucosal lymphoid nodules. A catarrhal esophagitis characterized by an excess of mucus in the esophagus and a dilation of submucosal glands was observed 2 DPI (Fig. 11). Hydropic degeneration and intranuclear inclusion bodies were observed in the mucosal cells 3 DPI. Pyknotic nuclear debris and edema were present in the adjacent submucosa. On the fourth and fifth DPI, spongiosis and microvesicles were observed in the mucosa; and pyknosis, marginated chromatin, and intranuclear inclusion bodies were present in adjacent keratinocytes (Fig. 12). Necrotic debris separated the submucosa and mucosa beneath

Figure 9. Liver 5 DPI, Trial I. Multiple pinpoint foci of necrosis and petechial hemorrhages are present in the liver

Figure 10. Liver 5 DPI, Trial I. Several ecchymotic and petechial hemorrhages are present in the liver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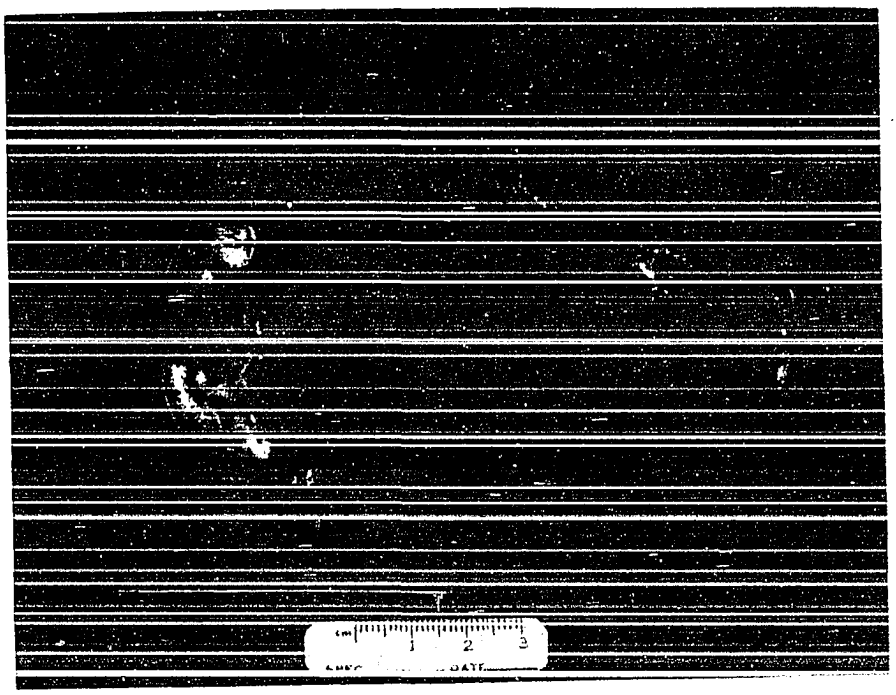
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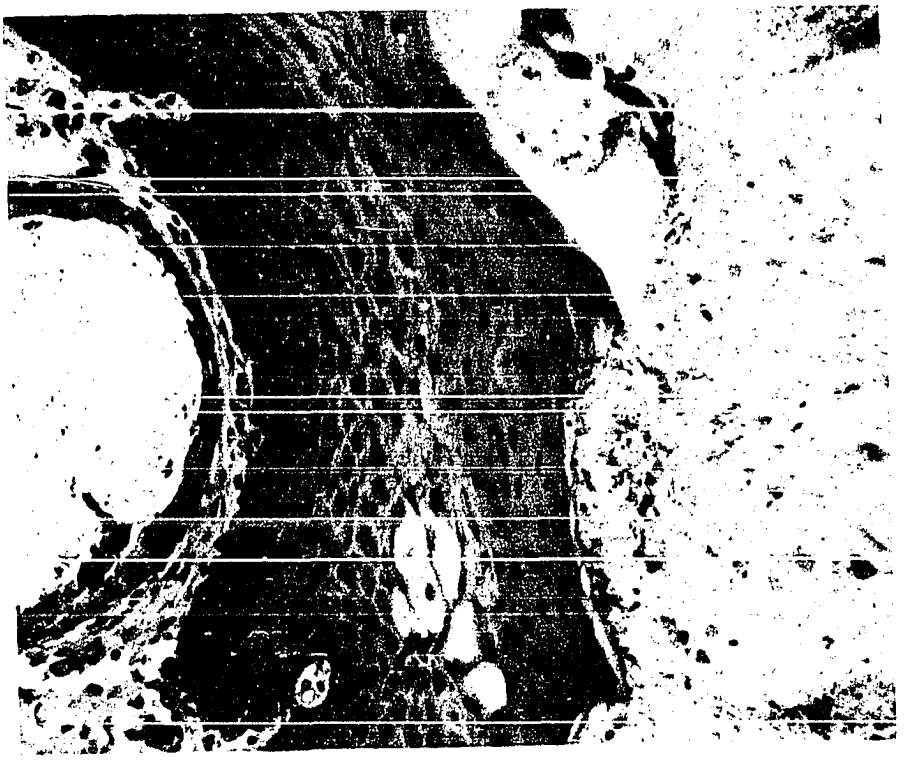
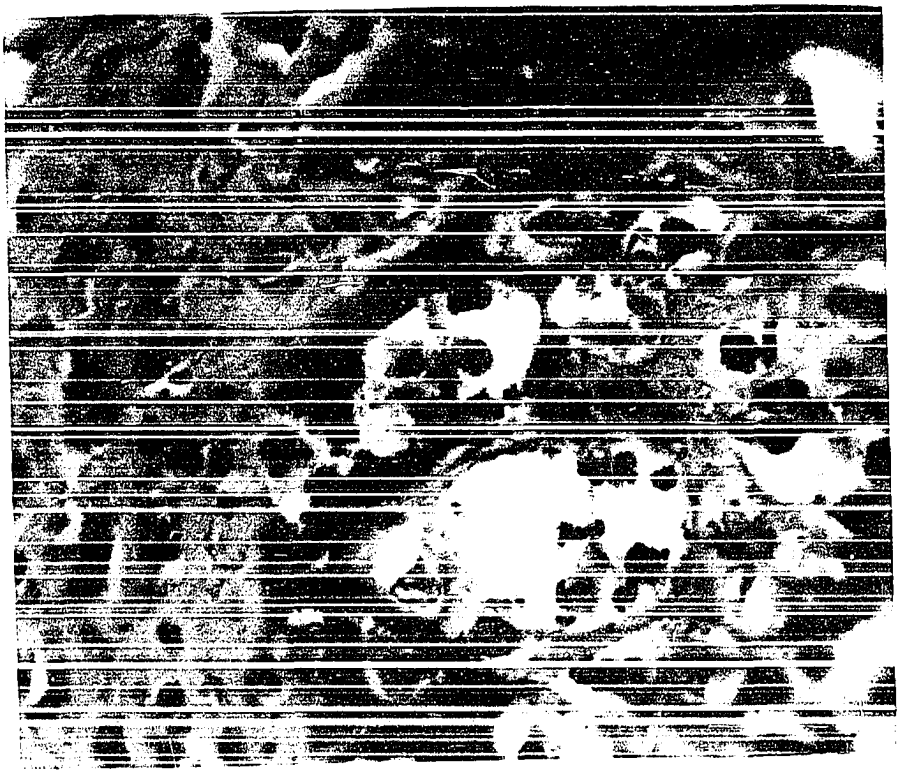
Figure 11. Esophagus 2 DPI, Trial I. A catarrhal esophagitis is characterized by excess mucus on the mucosa, dilation of submucosal glands, and intercellular edema in a few mucosal epithelial cells. H & E. x600

Figure 12. Esophagus 5 DPI, Trial I. Hydropic degeneration is present in the mucosal epithelial cells. A few cells have coalesced to form small vesicles. Chromatin margination and intranuclear inclusion bodies are present in the epithelial cells. Edema causes the submucosa to appear homogenous, and the nuclei of fibrocytes are pyknotic. H & E. x1,700





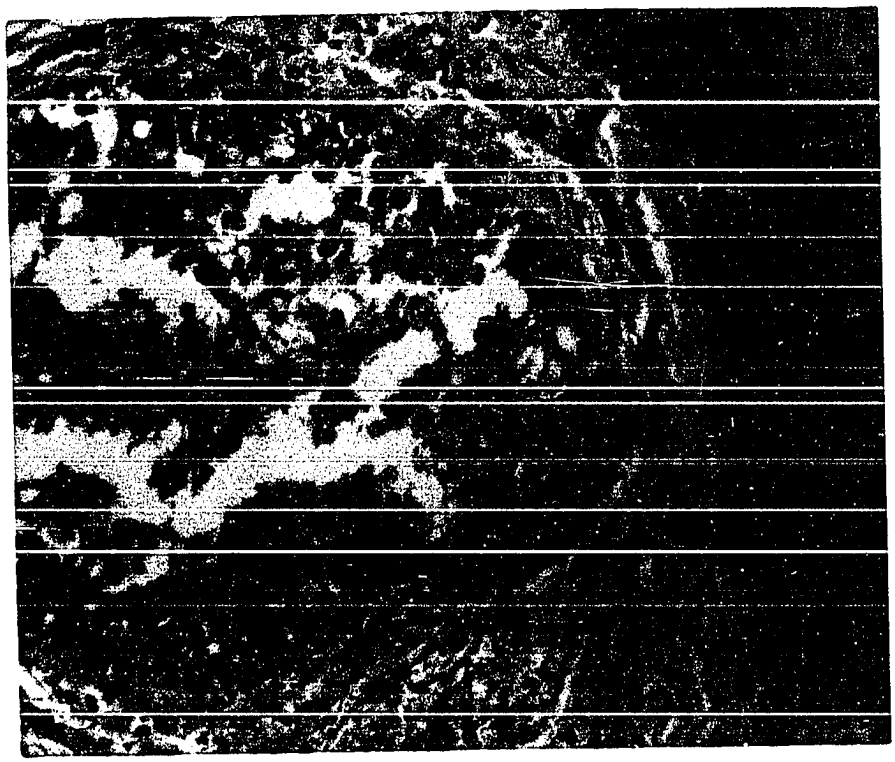
diphtheritic membranes. These membranes consisted microscopically of necrotic mucosa covered by a thick layer of fibrin.

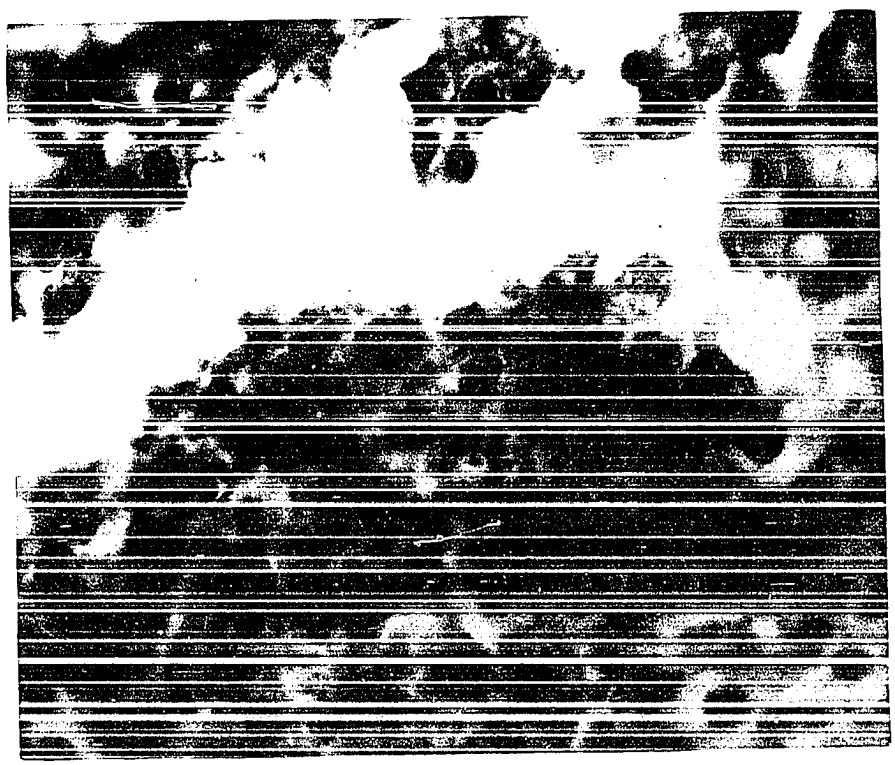
On the third DPI lesions were present in submucosal glands. Nuclei were no longer located basally, giving the cells a pseudostratified appearance. Sloughed cells were present in the lumen, and a few intranuclear inclusion bodies were noted in the remaining cells (Figs. 13 and 14). Hemorrhage and necrosis of submucosal periglandular tissue were evident. Intranuclear inclusion bodies were seen in a few of these connective tissue cells. Cytolysis and karyorrhexis were evident in most of the submucosal glandular cells on the fourth and fifth DPI. Cellular detritus and mucus filled many of the glandular lumens.

Necrosis of submucosal lymphoid nodules was observed 3 DPI and was easy to locate at the esophageal-proventricular junction where crypts of stratified squamous epithelium extend into lymphoid aggregates. Crypt epithelial cells were degenerate, and a few nuclei contained inclusion bodies. Inclusion bodies were occasionally noted in degenerate cells in the submucosa around the crypts. On the fourth and fifth DPI necrotic lymphoid detritus was usually obscured by hemorrhage, and many crypt epithelial cells were sloughed (Fig. 15).

Figure 13. Submucosal esophageal gland 3 DPI, Trial I. Normally the esophageal submucosal glands are lined by a tall simple columnar epithelium with basally located nuclei. Cytoplasmic vacuolation, nuclear pyknosis, and sloughed epithelial cells are present in the gland. Focal hemorrhage and pyknotic fibroblasts are present in the surrounding submucosa. H & E. x600

Figure 14. Submucosal esophageal gland 3 DPI, Trial I. Intranuclear inclusion bodies are in the epithelial cells. H & E. x1,700



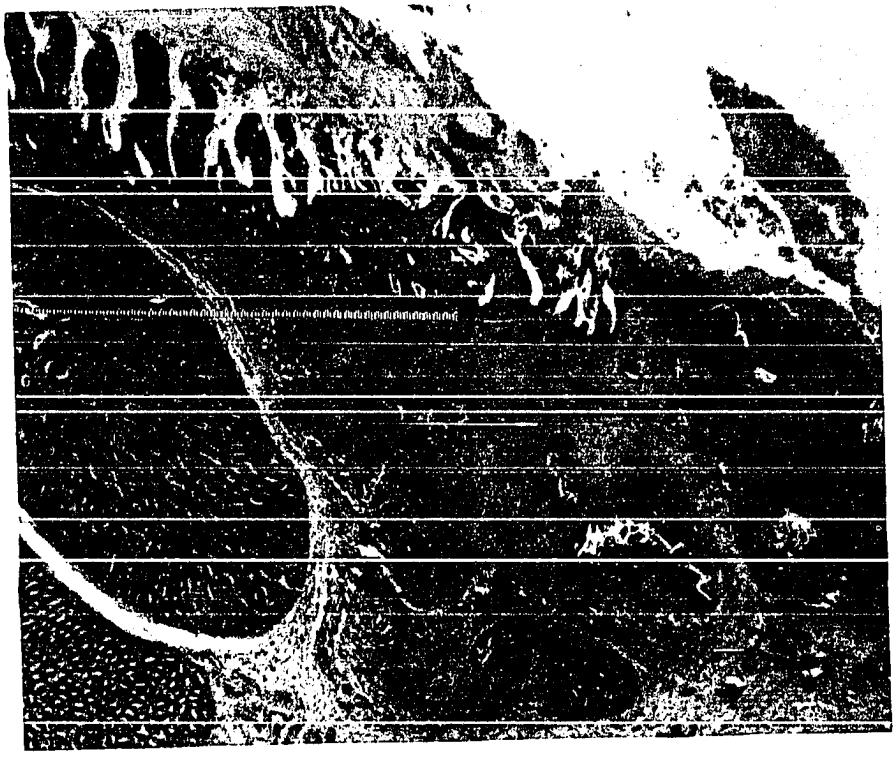


Large and small intestines Intestinal microscopic lesions of duck plague occurred in lymphoid aggregates (Meckel's diverticulum, annular bands, and scattered submucosal lymphoid nodules), lamina propria, and crypt epithelium. Although the intestinal villi become progressively shorter toward the posterior part of the intestine, this anatomical difference did not alter microscopic lesions in the small and large intestines. A mild catarrhal enteritis was detected in one duck examined on the second DPI. In ducks examined 3 DPI, cellular necrosis and edema were evident in the lamina propria. An occasional intestinal villous tip was swollen because of collections of dark nuclear debris and homogenous eosinophilic fluid in the lamina propria (Fig. 16). Lamina propria necrosis was more diffuse on the fourth and fifth DPI. Smooth muscle extending into some villi was contracted, and mucosal epithelium was thrown into irregular folds or sloughed (Fig. 17). Intranuclear inclusion bodies were present in degenerate cells in the lamina propria, and fibrin covered the villous tips.

Crypt epithelial lesions became apparent on the fourth DPI when epithelial necrosis was evident. Some cells were sloughed into the lumen, and remaining viable cells in the crypts of Leiberkühn were low cuboidal to squamous (Fig. 17). Intranuclear inclusion bodies, chromatin margination, and pyknosis were all evident in crypt epithelial cells (Fig. 18). Inclusion bodies were not detected in villous cells near the tip.

Figure 15. Esophageal-proventricular junction 4 DPI, Trial I. Necrosis of lymphocytes, fibrin, and hemorrhage are present in the submucosa. Crypt epithelium is degenerate, and fibrin is present on the mucosa. H & E. x100

Figure 16. Jejunal villus 3 DPI, Trial I. Dark nuclear debris and edema are present in the villous tip. H & E. x1,000



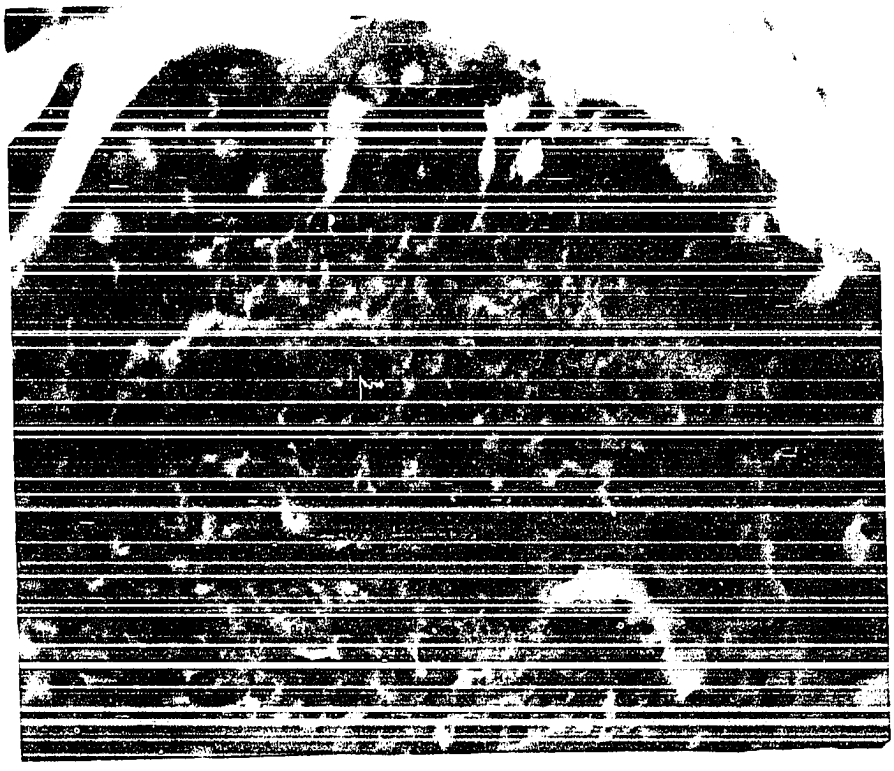
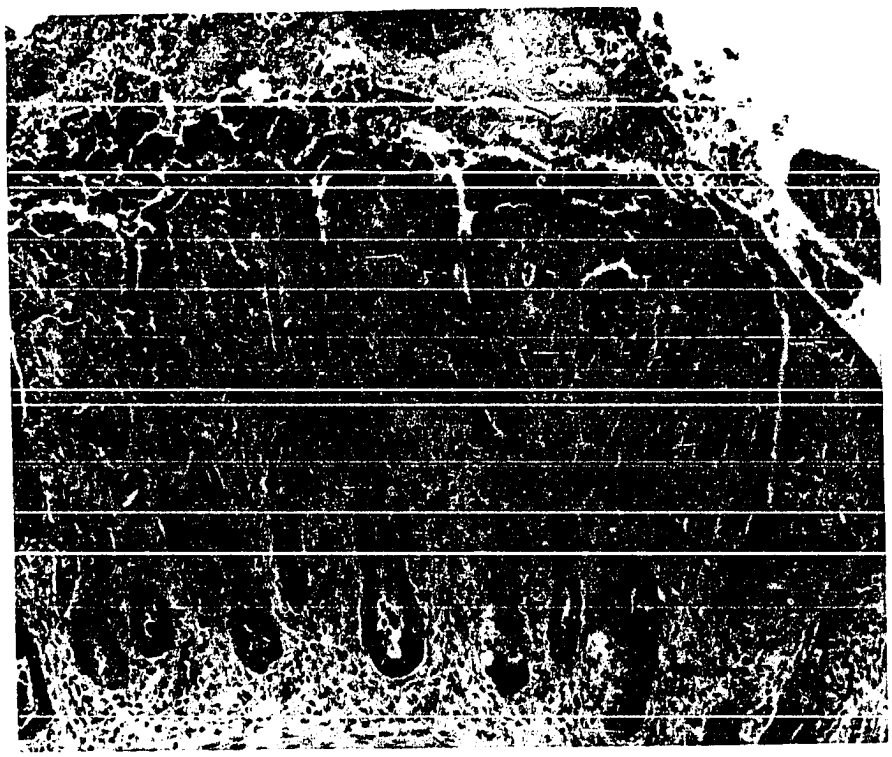
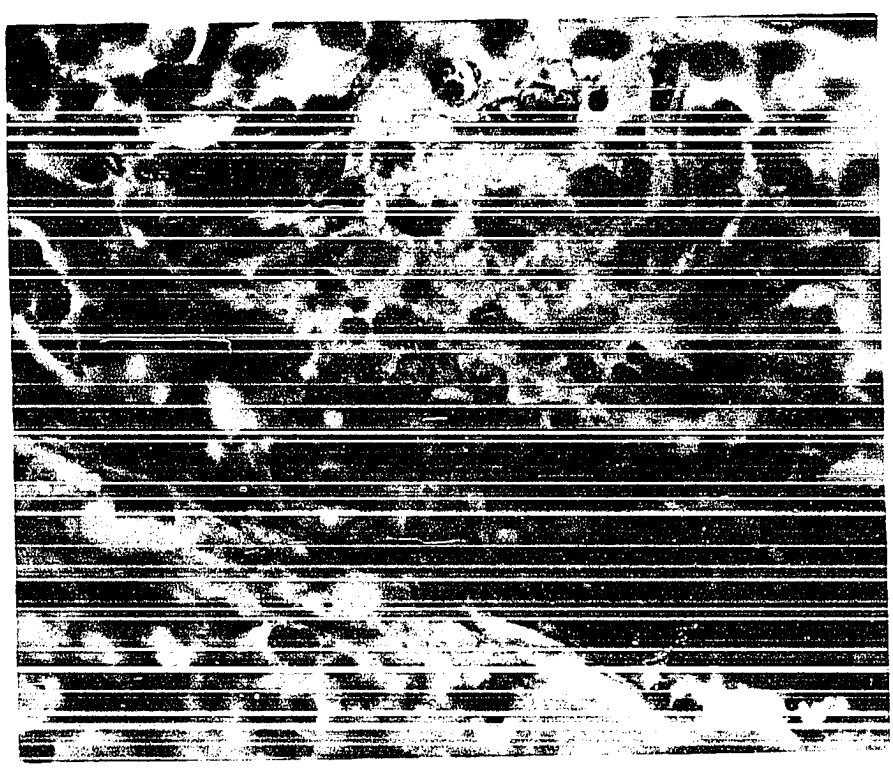


Figure 17. Ileum 4 DPI, Trial I. Villous smooth muscle is contracted, and the overlying mucosa is thrown into irregular folds. Fibrin is present on the tips of the villi. Sloughed epithelial cells are present in the crypts of Lieberkühn, and dark nuclear debris is present in the lamina propria. H & E. x250

Figure 18. Cecal crypt 4 DPI, Trial I. Intranuclear inclusion bodies are present in the crypt epithelium and in cells in the adjacent lamina propria. H & E. x1,700





Lymphocytic degeneration initiated similar lesions in the annular bands, Meckel's diverticulum, and scattered submucosal lymphoid nodules. Cytolysis of lymphocytes was first noted on the third DPI. Heterophils, erythrocytes, and edema were present between the degenerate lymphocytes. On the fourth and fifth DPI, diffuse necrosis had occurred in lymphoid tissue leaving only cellular detritus in the submucosa. This detritus was often obscured by hemorrhage. Large collections of fibrin were present in the submucosa and on the necrotic mucosa covering the lymphoid tissue (Fig. 19). Microscopically, the fibrin, necrotic mucosa, and degenerate submucosal tissue constituted the diphtheritic membranes over hemorrhagic necrotic tissue in annular bands, Meckel's diverticulum, and scattered submucosal lymphoid tissue.

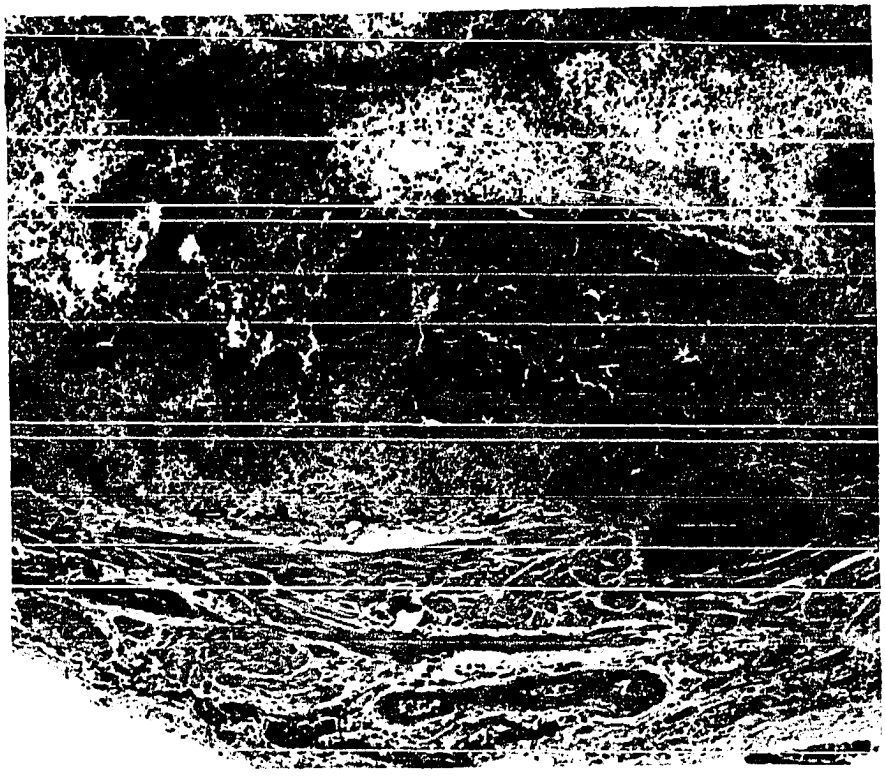
Histologically cloacal lesions were similar to esophageal lesions and were first noted on the fourth DPI. Hydropic degeneration, spongiosis, and heterophils were all observed in the stratified squamous mucosa 4 DPI. On the fifth DPI a focal fibrinous hemorrhagic necrotic cloacitis was observed. Fibrocytes in the submucosa were pyknotic and karyorrhetic. Diffuse necrosis and hemorrhage were observed in submucosal lymphoid nodules.

Spleen The spleens of two ducks examined 3 DPI were congested, and an increased number of heterophils were scattered throughout the red pulp. In the other duck necropsied 3 DPI, pyknotic and karyorrhetic lymphocytes were scattered throughout the periarteriolar lymphoid sheaths and germinal centers. By the fourth or fifth DPI, diffuse necrosis of lymphoid tissue (Fig. 20) and sinusoidal lining cells had occurred. Periarteriolar reticular sheaths were still intact, but they were degenerate. Pyknosis, chromatin margination, and intranuclear inclusion bodies were present in these reticular cells. Fibrin, erythrocytes, and cellular detritus surrounded these sheaths, but the arteriolar endothelium appeared normal (Fig. 21).

Bursa of Fabricius Lymphocytic depletion was present in the cortex and medulla of the bursal follicles on the third DPI. Some of the epithelial cells lining the bursa of Fabricius had been sloughed, and edema separated the connective tissue between the follicles (Fig. 22). Pyknosis and karyorrhexis were present in cortical and medullary lymphocytes on the fourth and fifth DPI (Fig. 23). Necrosis was more diffuse in the medullary lymphocytes, and the medulla often appeared microscopically as a cavity containing scattered nuclear remnants. Chromatin margination, intranuclear inclusion bodies, and cytoplasmic eosinophilia were seen in the epithelial cells separating the cortex and medulla (Fig. 24).

Figure 19. Annular band 4 DPI, Trial I. Diffuse necrosis has occurred in the submucosal lymphoid tissue. Fibrin and erythrocytes obscure the lymphoid detritus. H & E. x150

Figure 20. Splenic germinal center 4 DPI, Trial I. Cellular detritus and fibrin are all that remains of a necrotic splenic lymphocytic nodule. H & E. x1,000



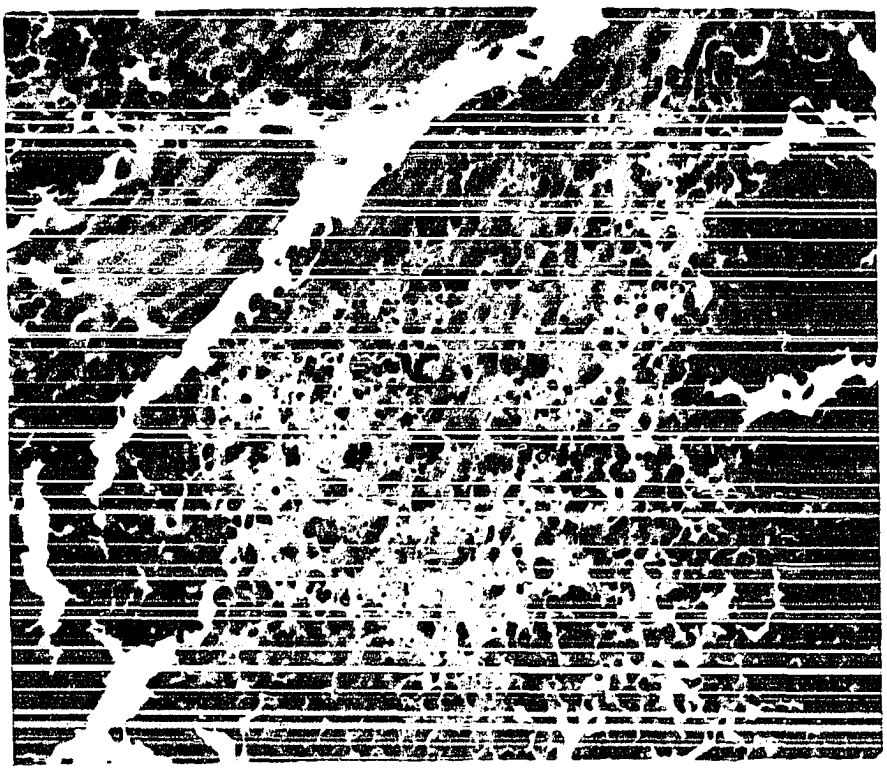
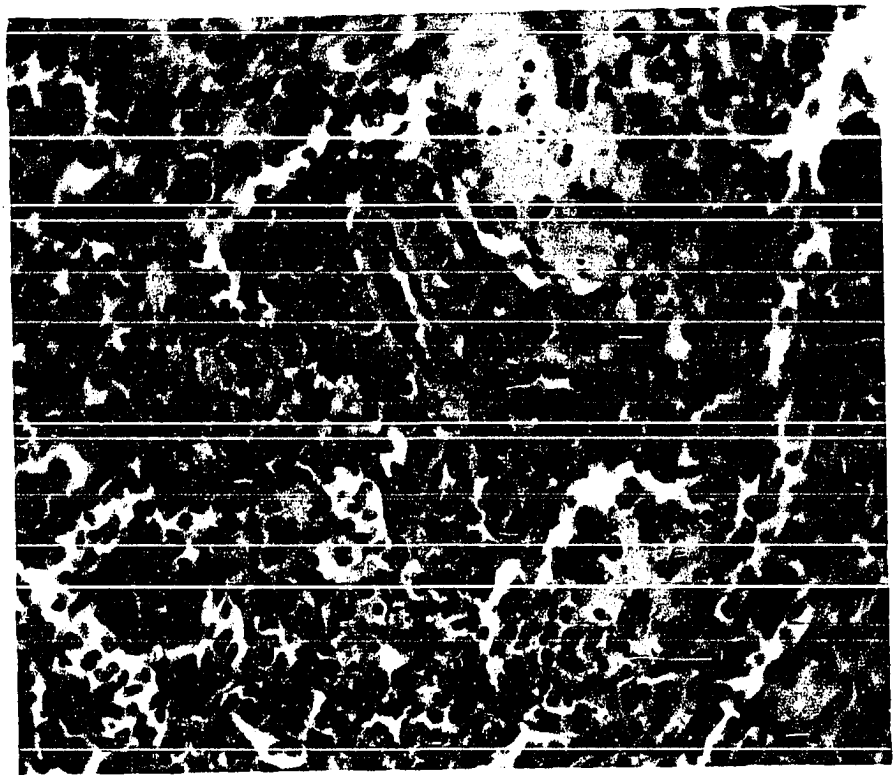


Figure 21. Spleen 4 DPI, Trial I. Arteriolar reticular sheath is present in the center. Endothelium lining the arteriole appears normal. Cytoplasm of the reticular cells is very eosinophilic; and pyknosis, chromatin margination, and intranuclear inclusion bodies are evident in the nuclei. H & E. x1,000

Figure 22. Bursa of Fabricius 3 DPI, Trial I. Lymphocytic depletion outlines the epithelial cells separating the cortex and medulla of a bursal follicle. Many columnar epithelial cells lining the bursal lumen have been sloughed. H & E. x250



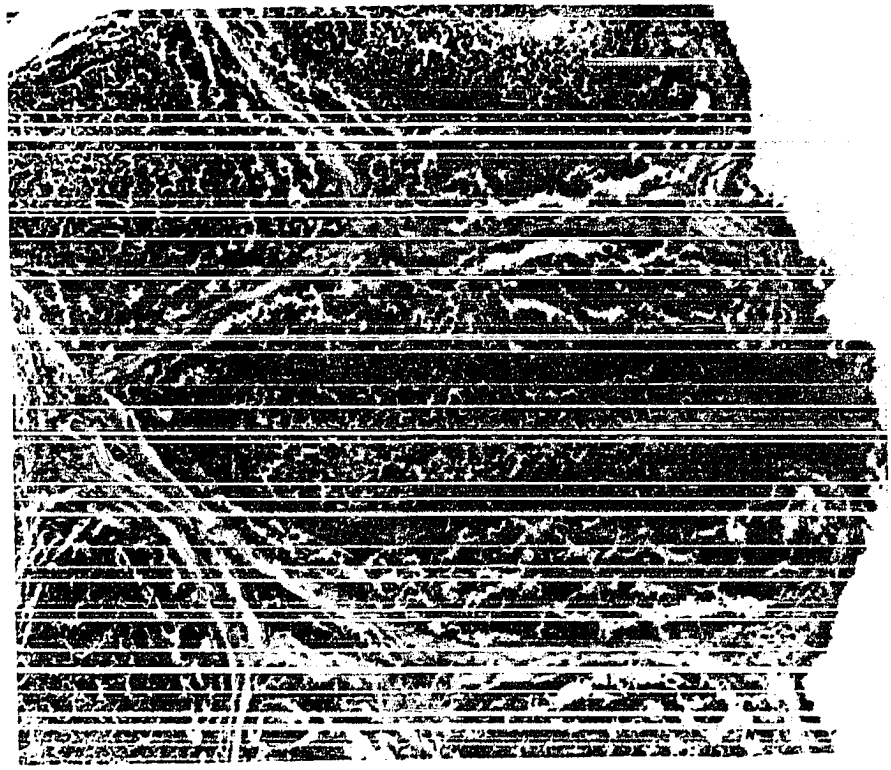
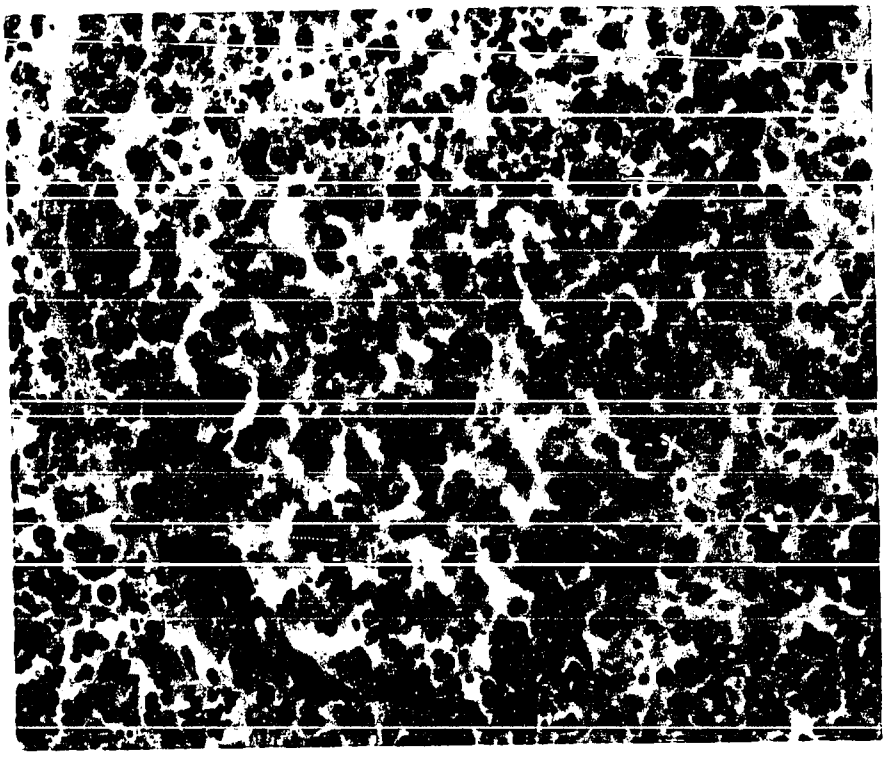
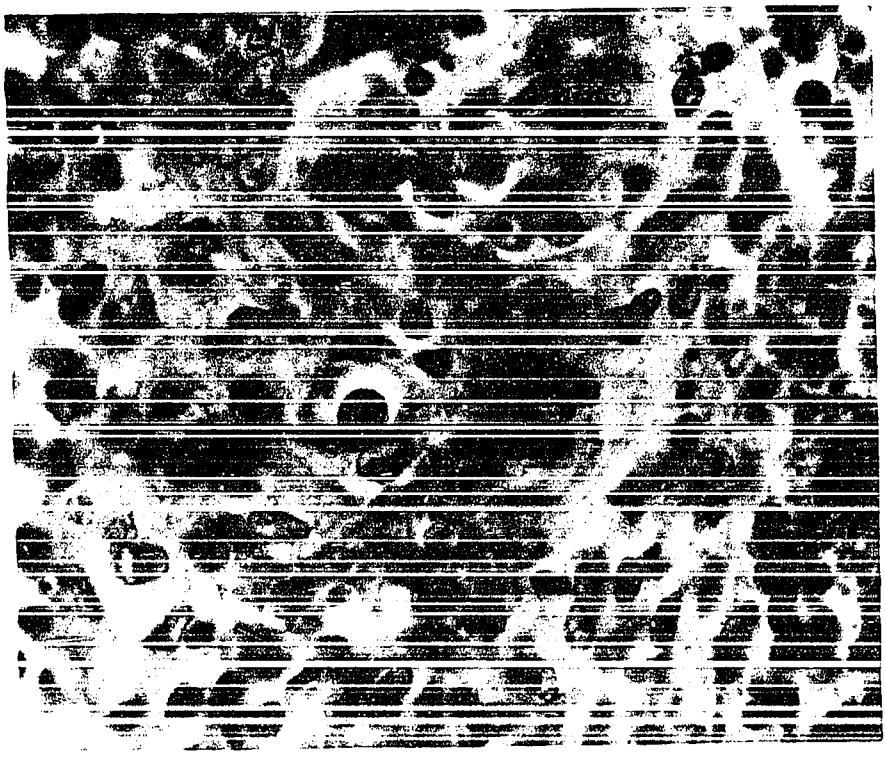


Figure 23. Bursa of Fabricius follicle 4 DPI, Trial I. Dark lymphocytic nuclear debris is present in the cortex and medulla. H & E. x1,000

Figure 24. Bursa of Fabricius 4 DPI, Trial I. Pyknotic lymphocytic nuclei are present in the cortex and medulla. Intranuclear inclusion bodies are present in the epithelial cells separating the cortex and medulla. H & E. x1,700



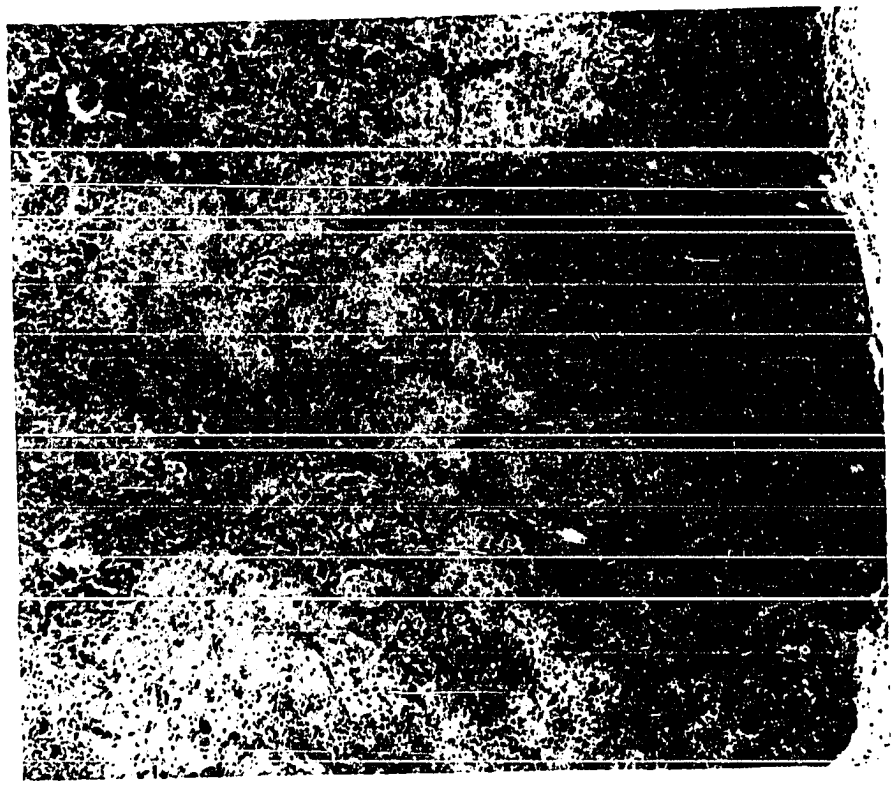


Thymus Lymphoid depletion and eosinophilic degeneration of cells in Hassel's corpuscles were seen in one duck examined 3 DPI. By the fourth and fifth DPI diffuse necrosis of medullary lymphocytes and depletion of cortical lymphocytes had occurred. Fibrin and cellular debris surrounded the degenerate Hassel's corpuscles (Fig. 25). Chromatin margination, intranuclear inclusion bodies, and eosinophilic granular cytoplasm were seen in Hassel's corpuscular cells (Fig. 26).

Pancreas Scattered foci of degeneration were seen in acinar, islet, and ductal cells of the pancreas of one duck examined 4 DPI. Focal pancreatic necrosis involving ductal epithelium, acinar cells, and islet cells was found in 5 of 13 ducks 5 DPI. Acinar cell degeneration was characterized by loss of apical and basal differential staining, followed by separation of the acinar cells, pyknosis, chromatin margination, and intranuclear inclusion bodies (Fig. 27). Islet cell involvement was more difficult to discern. Fine cytoplasmic granules and intranuclear inclusion bodies without marked chromatin margination were noted in affected cells (Fig. 28). Diffuse necrosis of islet cells was not seen. In ductal epithelium cytoplasmic eosinophilia, chromatin margination, and intranuclear inclusion bodies were seen in affected cells (Fig. 29).

Figure 25. Thymus 4 DPI, Trial I. Lymphocytic necrosis and eosinophilic debris are present in the medulla. H & E. x250

Figure 26. Thymic Hassel's corpuscle 5 DPI, Trial I. Chromatin margination and intranuclear inclusion bodies are present in the reticular cells. Cellular debris is present around the corpuscle. H & E. x1,700



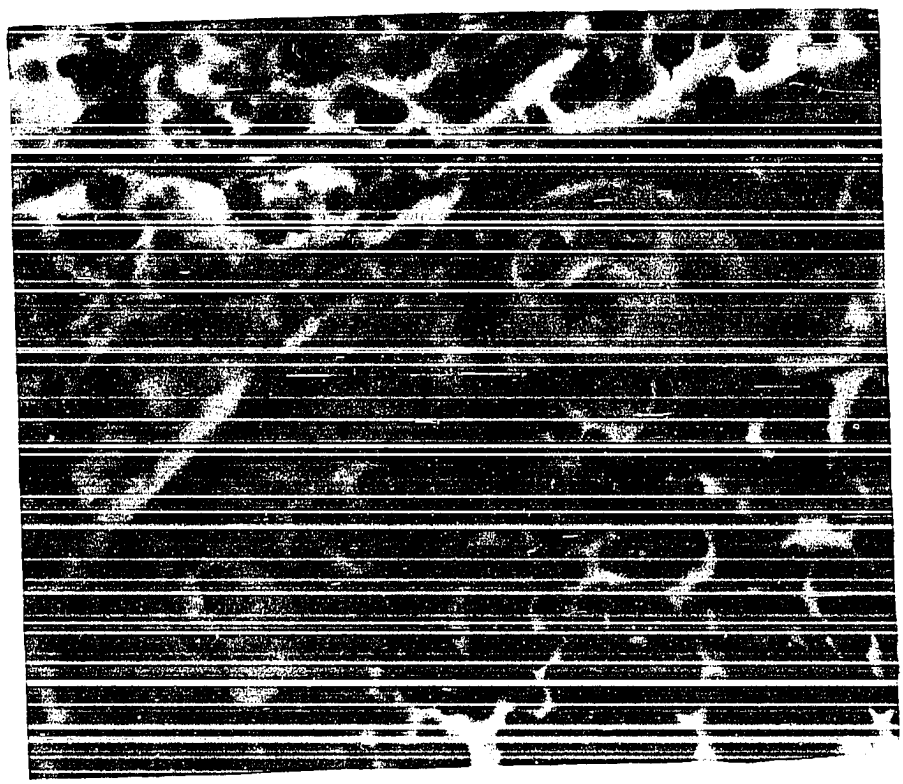
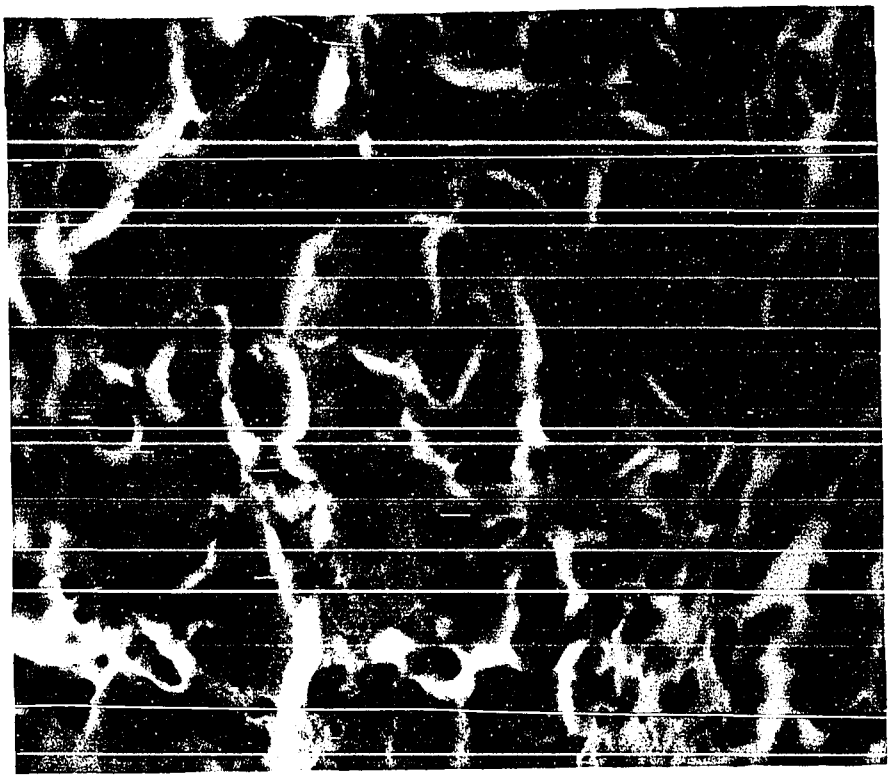
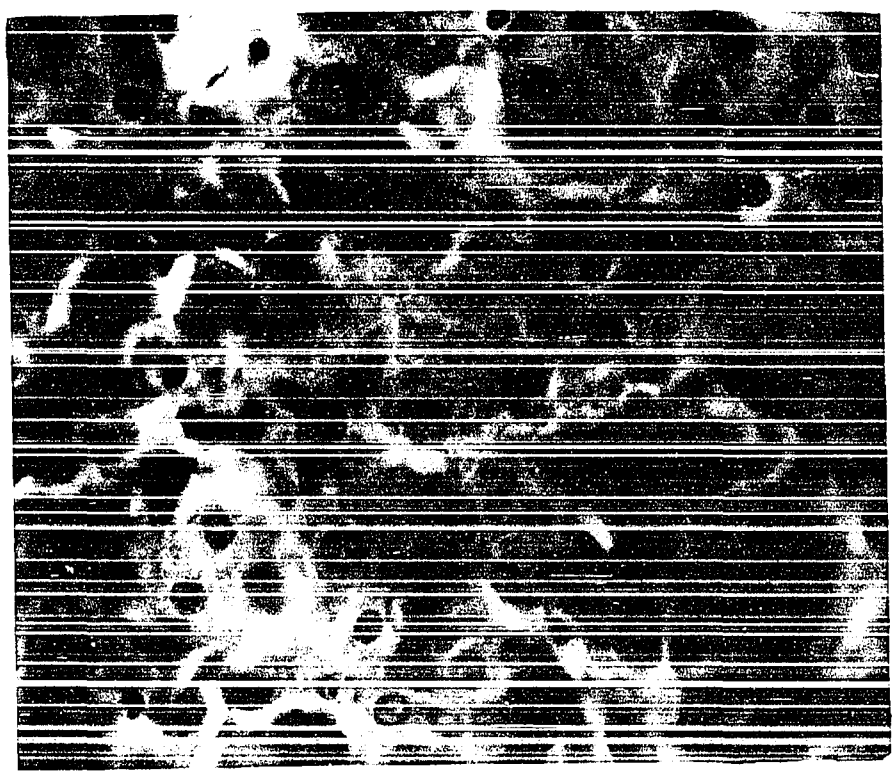


Figure 27. Pancreatic acinar cells 5 DPI, Trial I. The cells have lost their normal basal-apical differential staining property. Cytolysis, chromatin margination, and intranuclear inclusion bodies are present in some acinar cells. H & E. x1,700

Figure 28. Islet cells 5 DPI, Trial I. The cytoplasm of the islet cells is granular and contains fine vacuoles. Chromatin margination and intranuclear inclusion bodies are present in some cells. H & E. x1,700





Liver Hepatic microscopic lesions appeared on the third DPI. Hepatic sinusoids were dilated, congested, and contained scattered heterophils. Kupffer cells and hepatocytes were swollen. An occasional focus of hepatocytes could be detected with one large or numerous smaller fat vacuoles in the cytoplasm. Small nonzonal foci of hepatic necrosis were noted on the fourth DPI. Clusters of degenerate cells resembling syncytia were present in some necrotic foci (Fig. 30). Cytoplasm of hepatocytes adjacent to necrotic areas was eosinophilic and granular; and pyknosis, chromatin margination, karyorrhexis, and intranuclear inclusion bodies were discernible in the nuclei (Fig. 31). These degenerate cells became separated from hepatic cords, and adjacent sinusoids were congested. Focal areas of hepatic necrosis were more numerous on the fifth DPI, and diffuse areas of hemorrhage were present in some necrotic foci.

Bile duct degeneration occurred in the small and large bile ducts. Pyknosis, chromatin margination, and karyorrhexis were visible in cholangioles 4 DPI. In the larger bile ducts, epithelial cytoplasm lost its normal homogenous eosinophilic appearance, stained slightly basophilic, and was granular. Nuclei were no longer located basally and caused the epithelium to appear pseudostratified. Nuclear pyknosis, chromatin margination, karyorrhexis, and intranuclear inclusion bodies were seen (Fig. 32). Inflammatory cells were seldom present

Figure 29. Pancreatic duct 5 DPI, Trial I. Dark nuclear detritus is present in the surrounding connective tissue. Cytoplasm of the duct cells is granular, and chromatin margination and intranuclear inclusion bodies are present in the nuclei of ductal cells. H & E. x1,700

Figure 30. Liver 4 DPI, Trial I. A nonzonal focus of hepatic necrosis is present. Sinusoids around the focal necrosis are dilated and congested. One cluster of degenerate cells in the center appears as a syncytium. H & E. x1,000

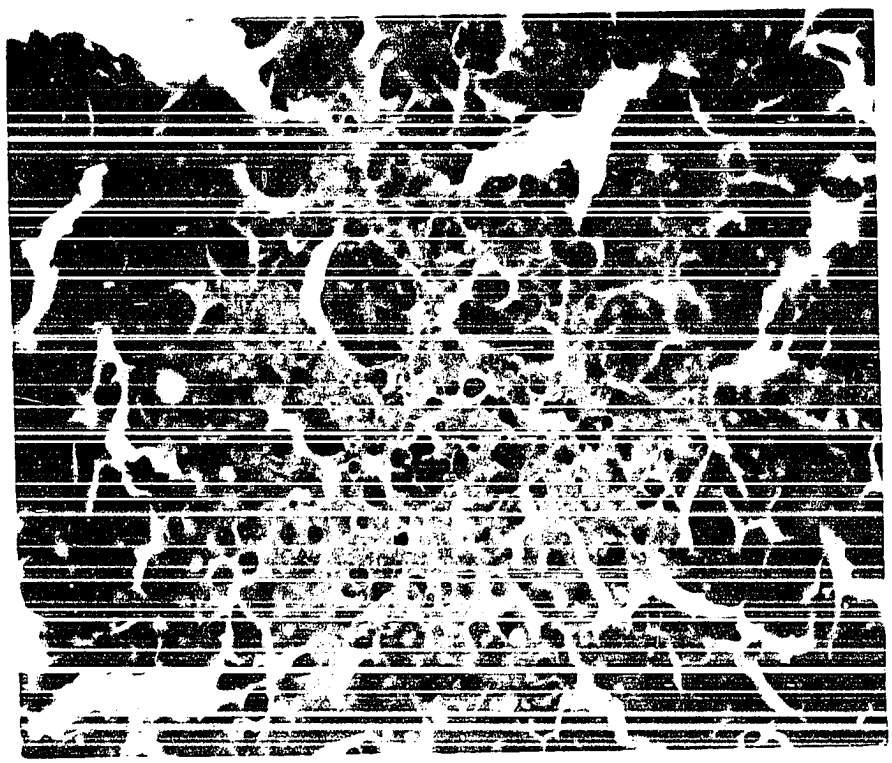
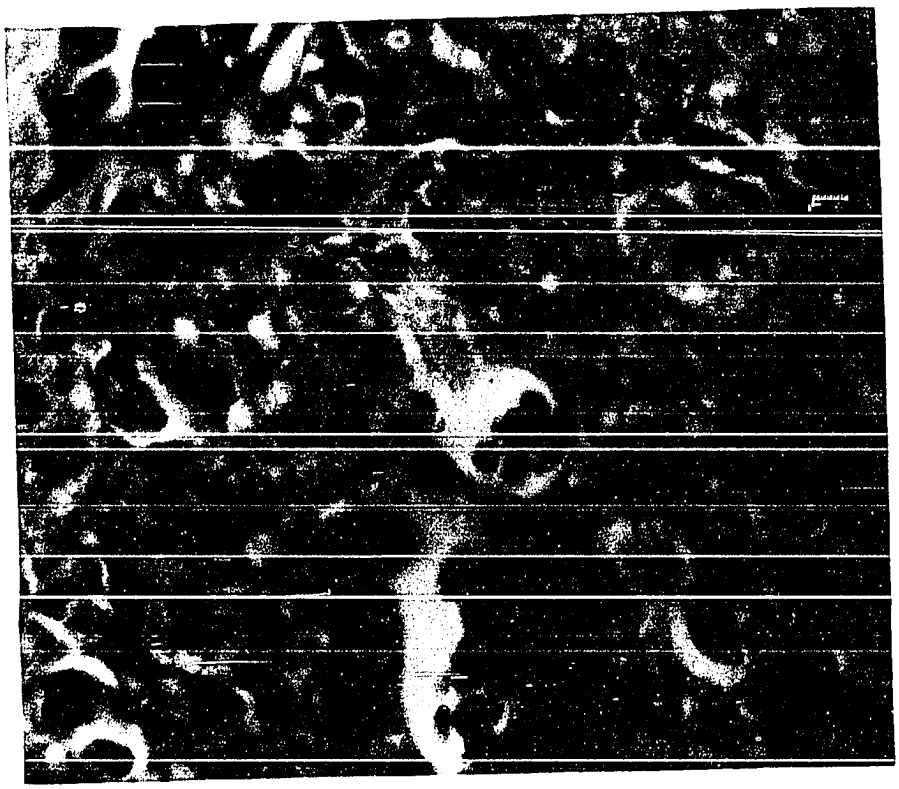
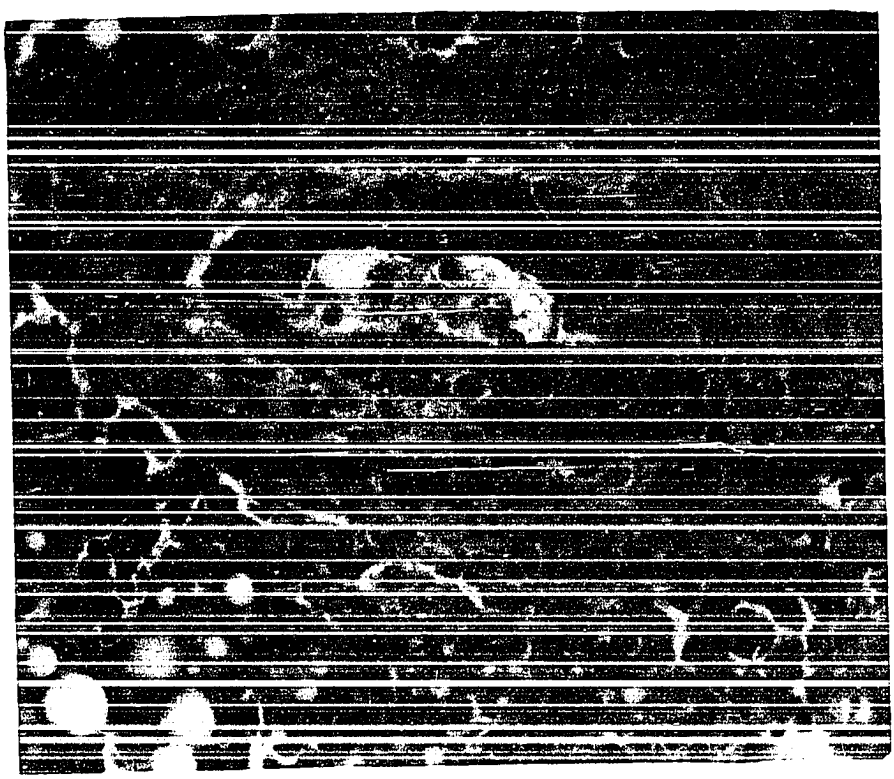


Figure 31. Liver 4 DPI, Trial I. The cytoplasm of the hepatocytes is vacuolated. Pyknosis, chromatin margination, and intranuclear inclusion bodies are present in some hepatocytes. H & E. x1,700

Figure 32. Liver 4 DPI, Trial I. Bile duct epithelium has lost its normal tall columnar appearance. The cytoplasm is granular, and nuclei are no longer located basally. Pyknosis, chromatin margination, and intranuclear inclusion bodies are present. H & E. x1,000





around degenerating bile ducts. Ductal necrosis was more diffuse on the fifth DPI.

Heart The only histologic lesions in the heart were hemorrhages and necrosis of small lymphoid nodules in myocardium the fourth and fifth DPI. Hemorrhage caused separation of bundles of myocardial fibers, and in most foci of hemorrhage a small necrotic lymphoid nodule was found (Fig. 33).

Respiratory tract Tracheal and bronchial lesions were found in each duck necropsied 3 DPI. Cytoplasmic vacuolation, loss of cilia, and nuclear pyknosis were observed in pseudostratified ciliated columnar cells. Scattered heterophils and erythrocytes could be seen between epithelial cells and in the submucosa of the trachea and bronchi (Fig. 34). On the fourth and fifth DPI, intranuclear inclusion bodies in a few epithelial cells, ulcers, submucosal hemorrhage, and necrosis of submucosal lymphoid nodules were seen in the trachea and bronchi (Fig. 35). Clusters of degenerate cells with intranuclear inclusion bodies which resembled syncytia were occasionally found in the peribronchial tissue. Intranuclear inclusion bodies were present in the syncytia (Fig. 36). Microscopic lesions appeared in the pulmonary parenchyma on the fourth DPI. Interstitial pneumonia was characterized by thickening of the air capillary walls and perivascular edema (Fig. 37). Intranuclear inclusion bodies were present in cells of the air

Figure 33. Heart muscle 4 DPI, Trial I. Hemorrhage is present between the myocardial fibers. A focus of pyknotic degenerate lymphocytes is present in the myocardium. H & E. x600

Figure 34. Trachea 3 DPI, Trial I. Hemorrhage is present in the submucosa. Pyknosis, cytoplasmic vacuolation, and stratification are present in the tracheal epithelium. H & E. x1,000

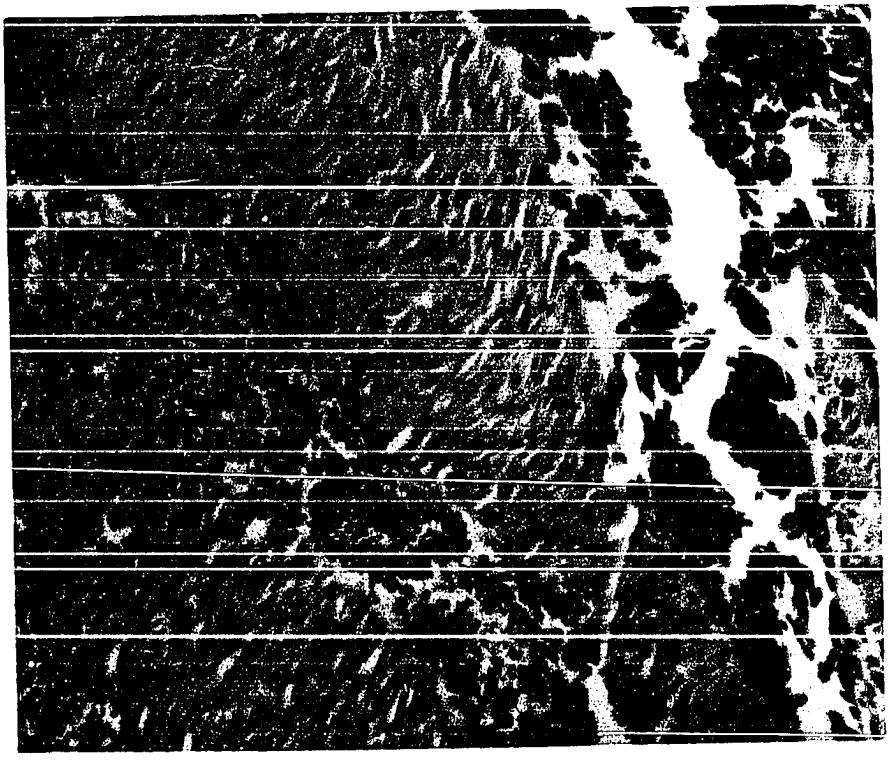
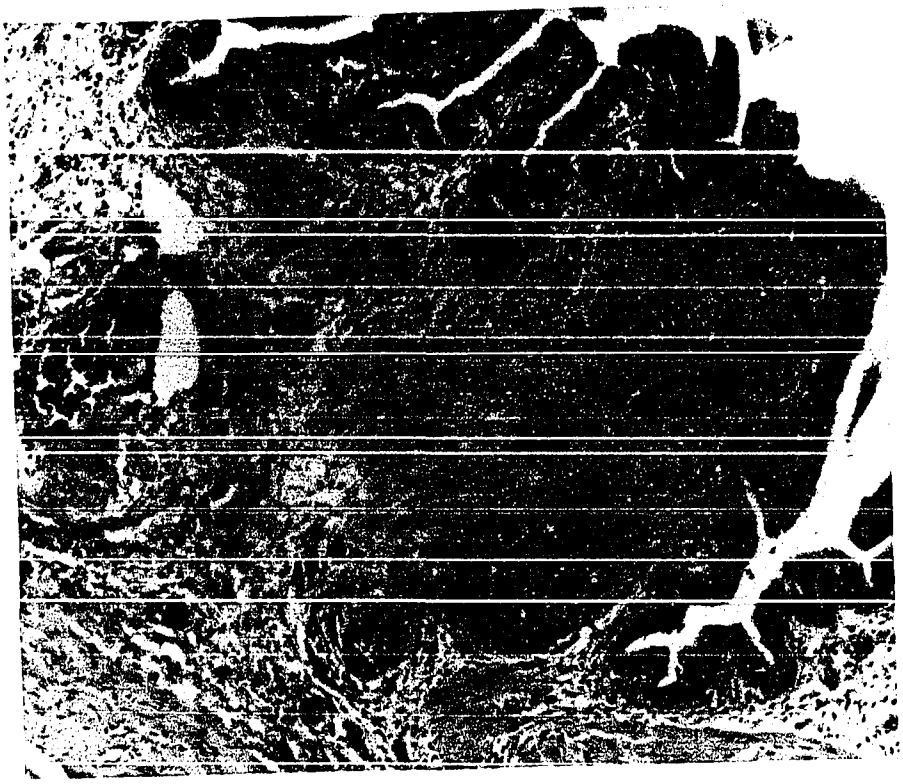
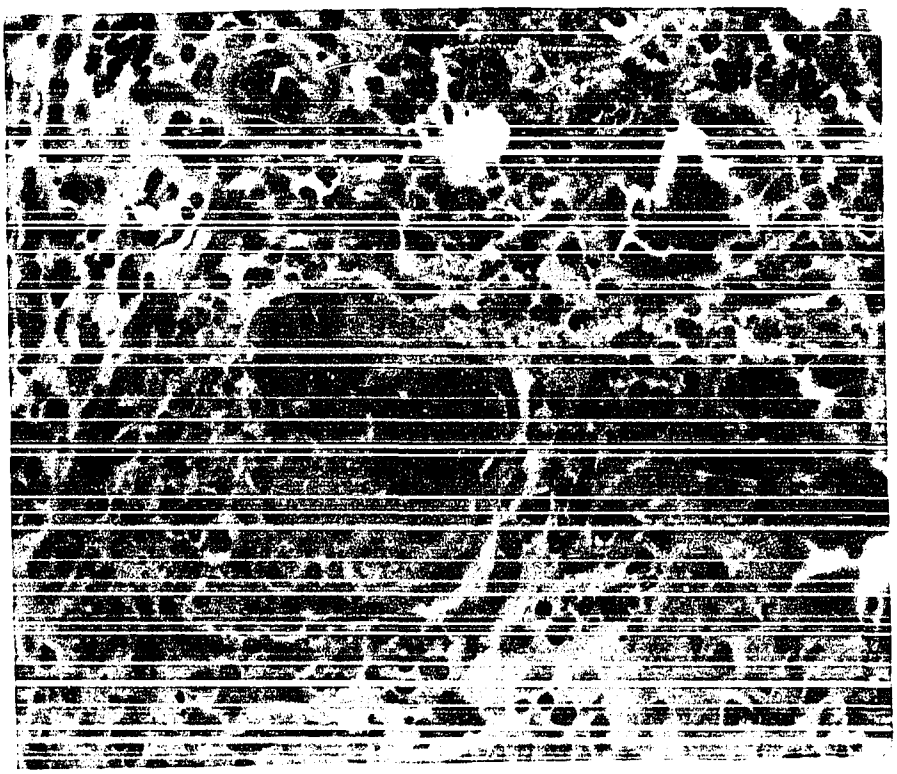


Figure 35. Bronchus 4 DPI, Trial I. A necrotic lymphoid nodule and hemorrhage are present in the submucosa. H & E. x250

Figure 36. Peribronchial tissue 5 DPI, Trial I. The syncytium contains intranuclear inclusion bodies. H & E. x1,000





capillaries (Fig. 38). A similar interstitial pneumonia was present in 10 of 13 ducks examined 5 DPI.

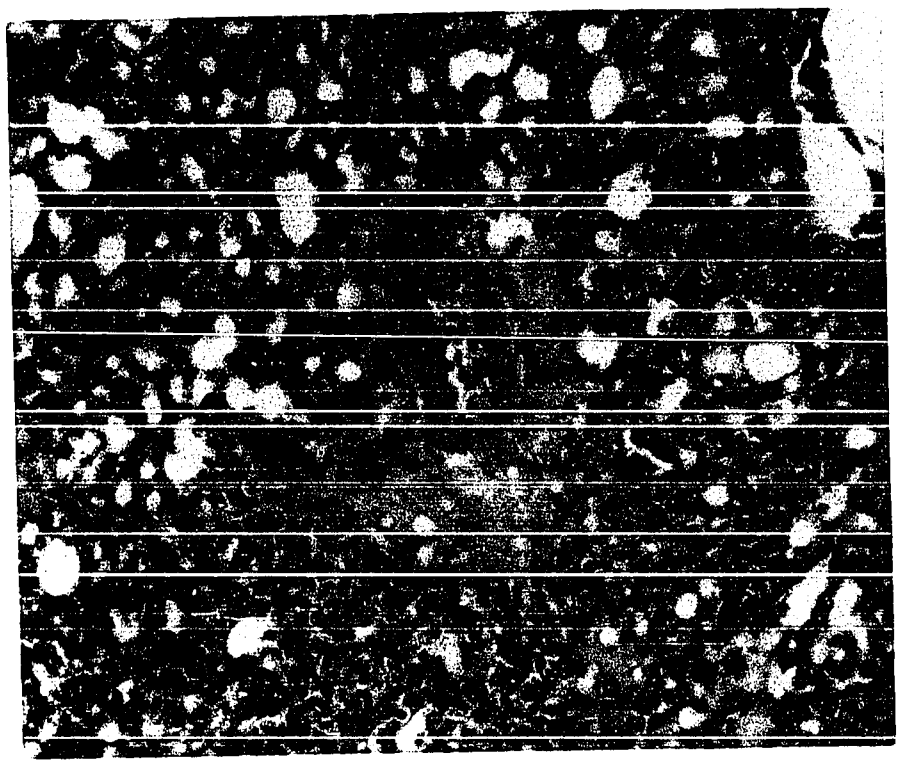
Adrenal gland Scattered foci of adrenal gland degeneration were found in the adrenal cortex on the fourth and fifth DPI. Cytoplasmic vacuolation, pyknosis, chromatin margination, and intranuclear inclusion bodies were seen in cortical cells (Fig. 39). Occasionally focal hemorrhages were found.

Bone marrow Histologic lesions were present in 2 of 3 ducks 4 DPI and 10 of 13 ducks 5 DPI. Necrosis was evident in all cell types of the bone marrow, and intranuclear inclusion bodies were present in the nuclei (Fig. 40). Osteoblasts lining spicules of osteoid near the epiphyseal line were degenerate, and many were missing. Osteoclasts were degenerate, contained intranuclear inclusion bodies, and were out of Howship's lacunae (Fig. 41).

Kidney Renal lesions were seen in 6 of 13 ducks examined 5 DPI. Dark nuclear detritus and hemorrhage were seen in the connective tissue surrounding the ureteral epithelium (Fig. 42). Pyknosis, chromatin margination, and intranuclear inclusion bodies were present in ureteral epithelium (Fig. 43).

Figure 37. Lung 4 DPI, Trial I. The walls of the air capillaries are thickened, and edema is present around the artery in the center. H & E. x600

Figure 38. Lung 4 DPI, Trial I. Intranuclear inclusion bodies are present in air capillary cells. H & E. x1,700



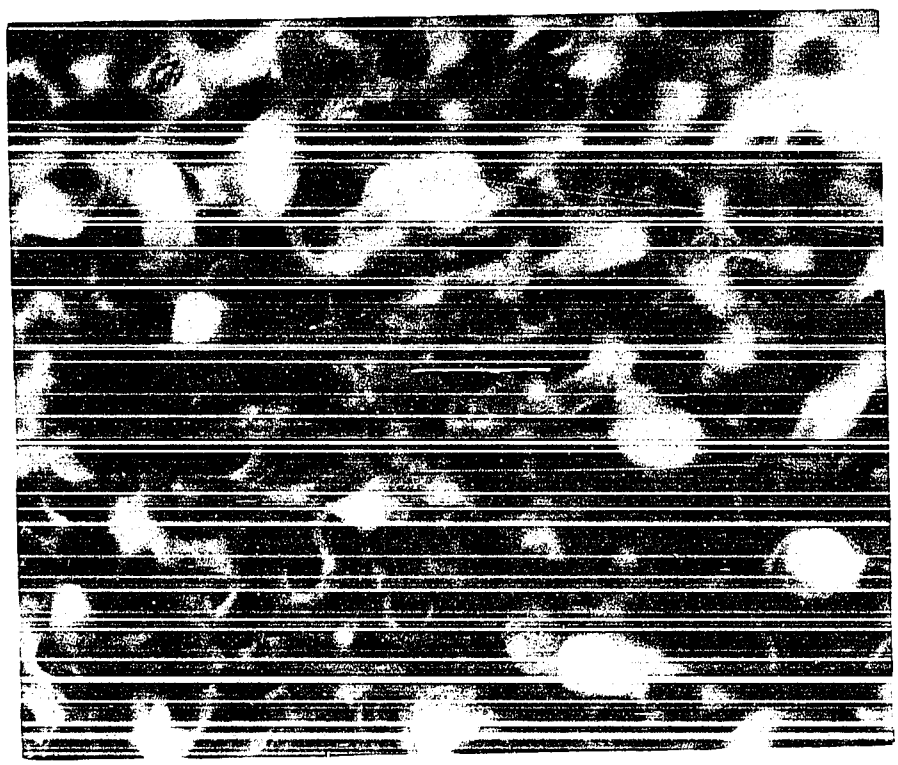
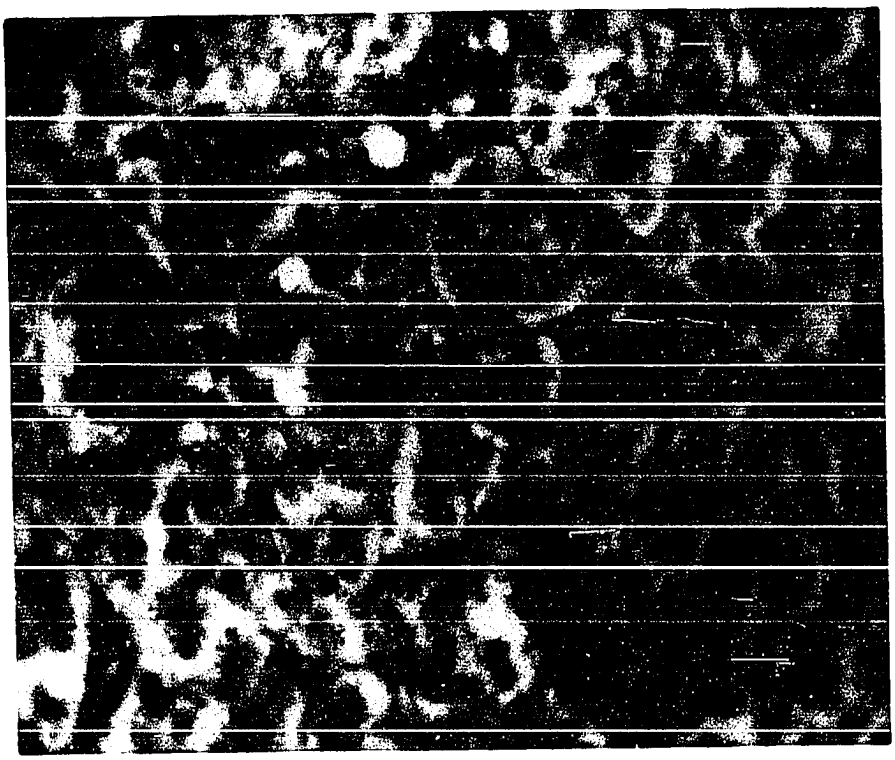


Figure 39. Adrenal gland 5 DPI, Trial I. Focal degeneration characterized by pyknosis, chromatin margination, intranuclear inclusion bodies, and cytoplasmic vacuolation is present in the adrenal cortex. H & E. x1,000

Figure 40. Bone marrow 5 DPI, Trial I. Necrosis and hemorrhage are present in the bone marrow. Osteoblasts are not present on the spicules of osteoid. H & E. x600



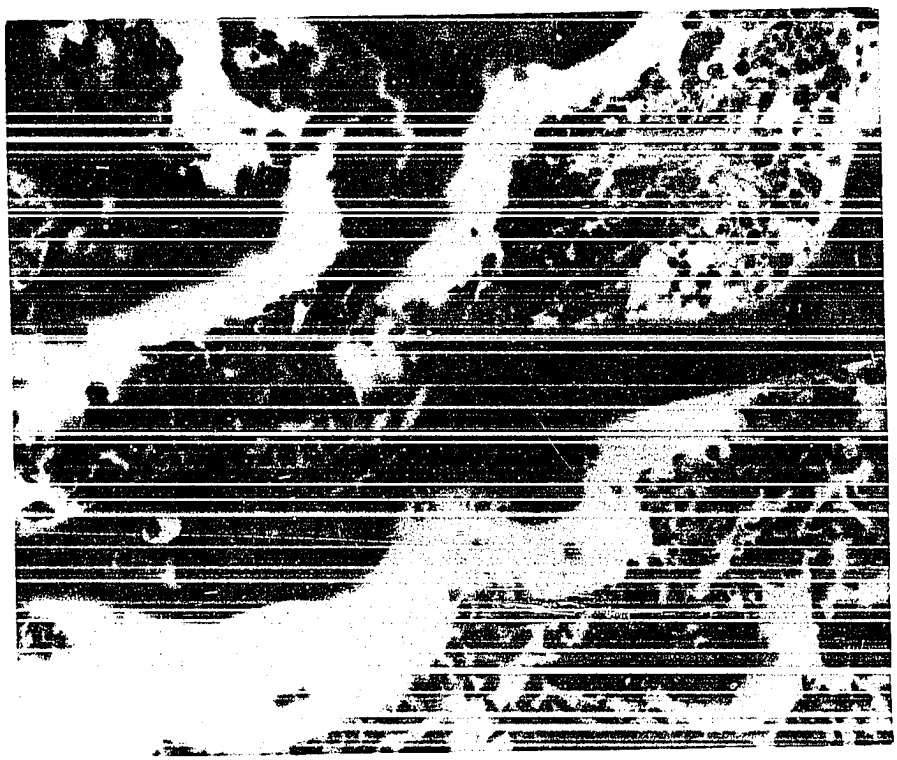


Figure 41. Bone marrow 5 DPI, Trial I. Osteoblasts lining the bony spicules are necrotic, and some are missing altogether. Osteoclasts are degenerate, and their nuclei are pyknotic. H & E. x600

Figure 42. Ureter 5 DPI, Trial I. Dark nuclear debris and hemorrhage are present in the connective tissue. Pyknosis is present in the ureteral epithelium. H & E. x600

Eye Focal corneal lesions were seen in 4 of 13 ducks 5 DPI. Anterior corneal epithelium in these foci contained vacuolated cytoplasm. Inclusion bodies were not seen in these cells (Fig. 44). The substantia propria was edematous.

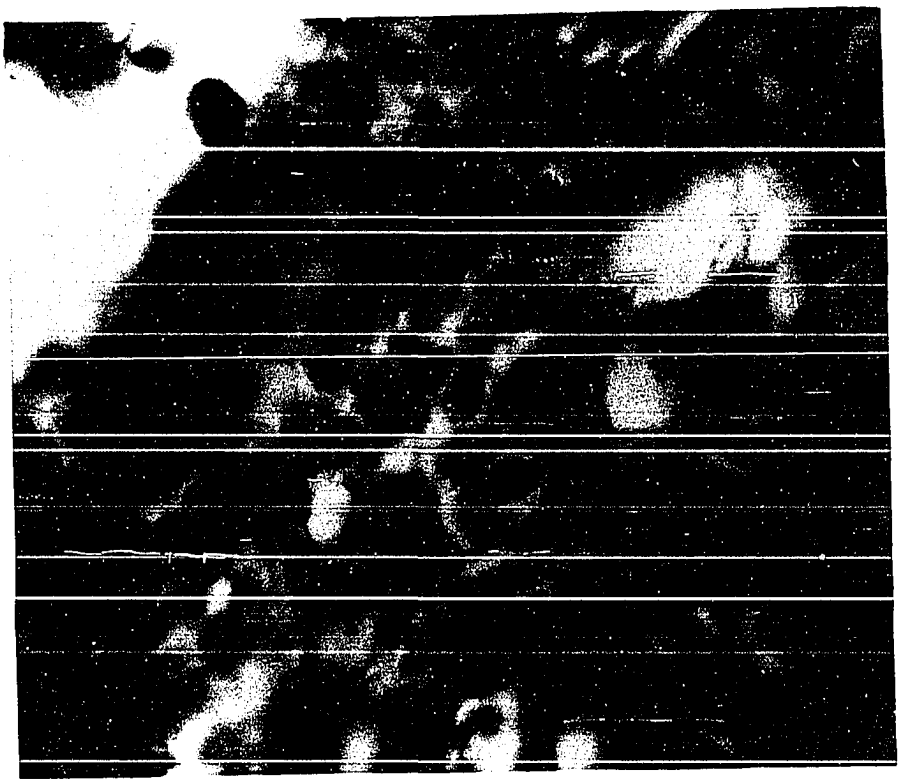
Fluorescent-antibody stained tissue sections (FASTS)

A summary of tissues which contained viral antigens is given in Table 1, and blocking techniques demonstrated the fluorescence observed in FASTS was specific for DPV antigens. Small foci of viral antigens were initially seen in the esophageal and cloacal mucosa 2 DPI. The third and fourth DPI large foci of cells containing viral antigens were seen in the mucosal epithelium, submucosal fibrocytes (Fig. 45), submucosal lymphocytes, and submucosal glands of the esophagus. Epithelial cells in the crypts of Lieberkühn and macrophages and fibrocytes in the lamina propria of the small and large intestines also contained viral antigens (Fig. 46).

Viral antigens were initially seen in parenchymatous organs 2 DPI. Scattered small foci of viral antigens were seen in cells of the periarteriolar reticular sheath. Larger amounts of antigen were present in the reticular sheaths 3 DPI, and smaller amounts were seen in splenic sinusoidal lining cells (Fig. 47), germinal centers, and periarteriolar lymphoid sheaths. Most splenic parenchymal cells contained viral antigens on the fourth and fifth DPI (Fig. 48). In the

Figure 43. Ureteral epithelium 5 DPI, Trial I. Intranuclear inclusion bodies, chromatin margination, and cytoplasmic vacuoles are present in the epithelium. H & E. x1,700

Figure 44. Cornea 5 DPI, Trial I. Cytoplasmic vacuoles are present in the small elevated foci of anterior corneal epithelium. Edema in the substantia propria causes it to appear homogenous. H & E. x600



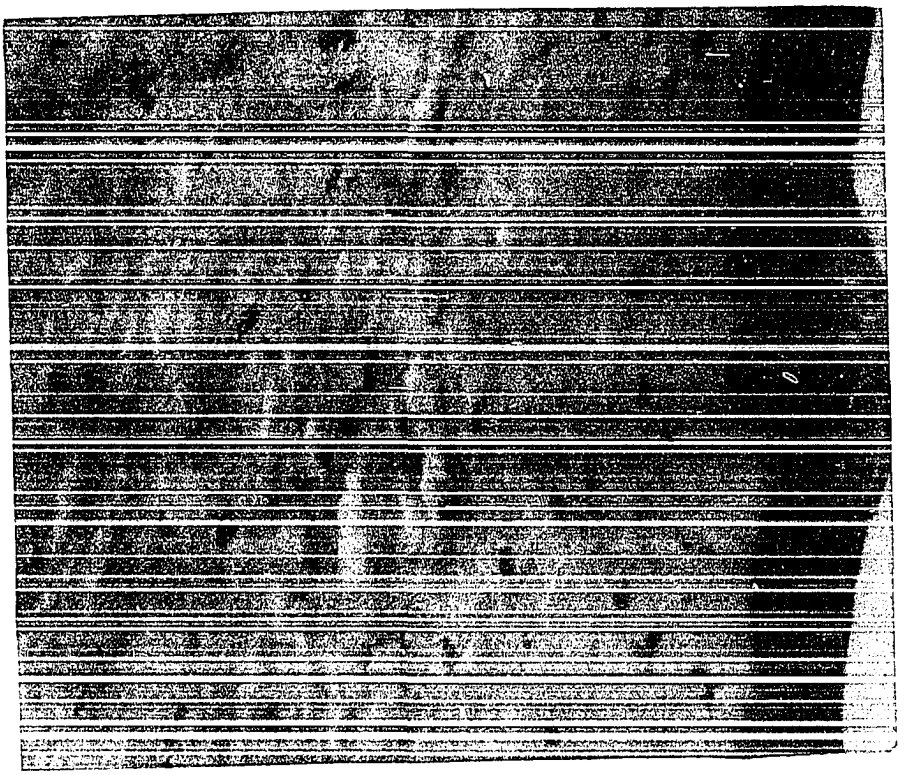


Table 1. Viral antigens detected in tissues

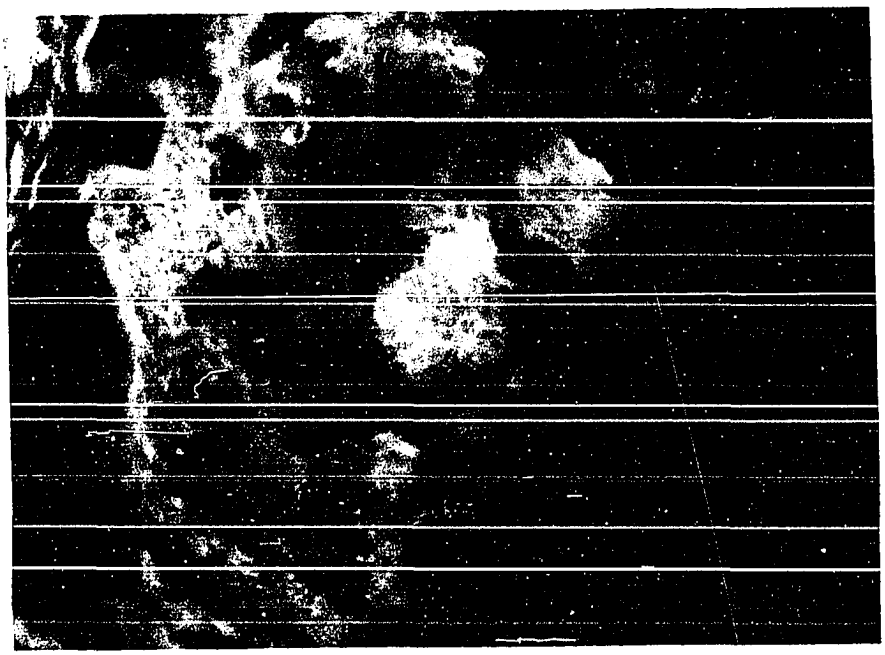
Tissue	Day postinoculation				
	1	2	3	4	5
Esophagus and cloaca					
Mucosa	-	+	++	+++	+++
Submucosal lymphoid nodules	-	-	++	+++	+++
Submucosal fibrocytes and macrophages	-	-	+	++	+++
Esophageal submucosal glands	-	-	+	+++	+++
Proventriculus and ventriculus					
Mucosa	-	-	-	-	-
Lymphoid nodules	-	-	-	++	++
Small and large intestines					
Villous epithelium	-	-	-	-	-
Crypt epithelium	-	-	+	+++	+++
Connective tissue and macrophages in lamina propria	-	-	+	++	++
Smooth muscle	-	-	-	-	±
Vascular endothelium	-	-	-	-	-
Kidney					
Ureteral epithelium	-	-	-	+	+
Glomeruli	-	-	-	-	-
Tubular epithelium	-	-	-	-	±
Spleen					
Germinal centers	-	-	+	+++	+++
Periarteriolar lymphoid sheath	-	-	+	+++	+++
Periarteriolar reticular sheath	-	+	+++	+++	+++
Vascular endothelium	-	-	-	-	-
Liver					
Hepatocytes	-	-	+	+++	+++
Bile duct epithelium	-	-	+	++	+++
Kupffer cells	-	-	++	+++	+++
Fibrocytes	-	-	+	+	+++

Adrenal glands					
Cortical cells	-	-	+	+	++
Medullary cells	-	-	-	-	-
Bursa of Fabricius					
Medullary lymphocytes	-	-	-	++	+++
Cortical lymphocytes	-	-	-	++	+++
Reticular cells	-	-	+	+++	+++
Heart					
Myocardium	-	-	-	-	-
Lymphoid nodules	-	-	+	+	++
Trachea					
Epithelium	-	-	++	++	++
Fibrocytes	-	-	++	++	++
Lung					
Bronchial epithelium	-	-	++	++	++
Peribronchial lymphocytes	-	-	++	++	+++
Fibrocytes	-	-	++	++	+++
Air capillary cells	-	-	++	++	+++
Brain					
Cerebellum, cerebrum, brain stem	-	-	-	-	-
Thymus					
Epithelial cells separating cortex and medulla	-	-	+	++	++
Lymphocytes	-	-	-	++	++

-	No viral antigens detected
±	Traces of viral antigen in a few cells
+	Viral antigens in individual cells
++	Foci of cells contained viral antigens
+++	Diffuse distribution of viral antigens

Figure 45. Cloaca 4 DPI, Trial I. Cells in the basal layer and stratum spinosum of the cloacal mucosa contain viral antigens. Fibrocytes in the submucosa also contain viral antigens. FASTS. x450

Figure 46. Ileum 4 DPI, Trial I. Viral antigens are present in the crypt epithelium. FASTS. x450



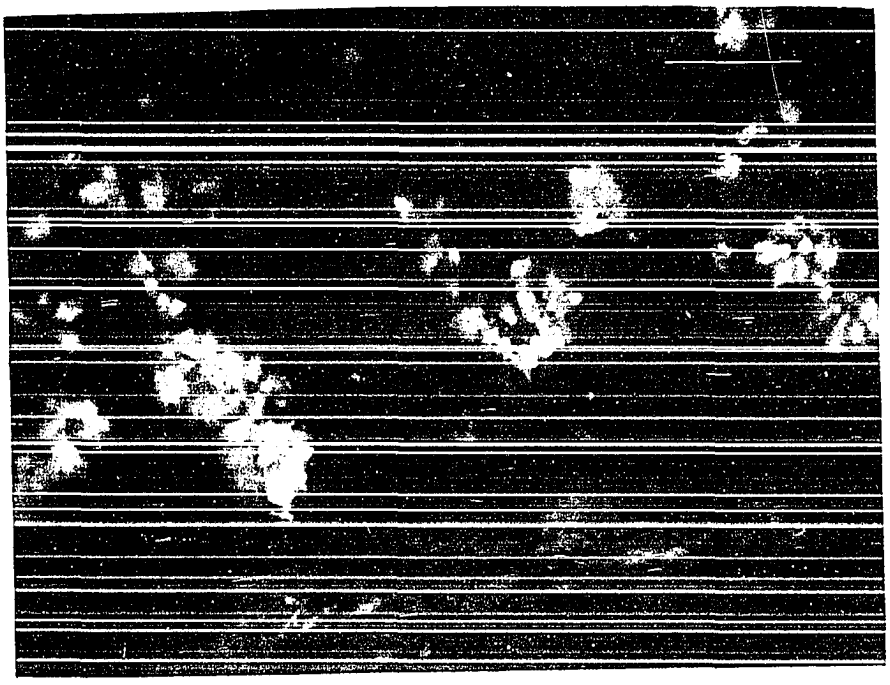
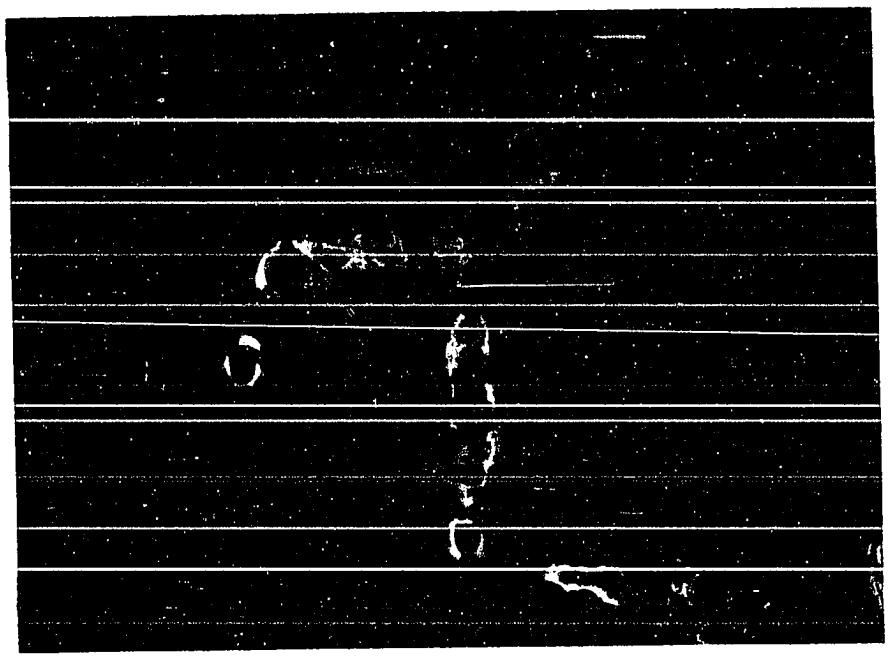
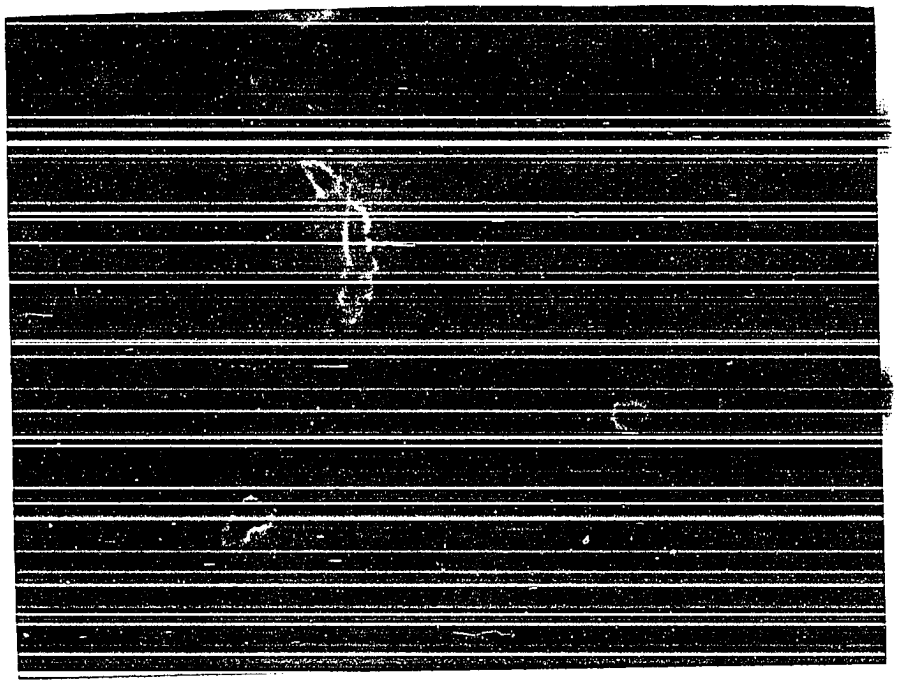


Figure 47. Spleen 3 DPI, Trial I. Small amounts of viral antigen are present in the periarteriolar reticular sheath and cells lining the sinusoids. FASTS. x450

Figure 48. Spleen 4 DPI, Trial I. Large amounts of viral antigen are present in the periarteriolar reticular sheaths and cells lining the splenic sinusoids. FASTS. x300





thymus and bursa of Fabricius antigen was initially found in epithelial cells separating the cortex and medulla of the bursal follicles and in cells of Hassel's corpuscles 3 DPI. These cells also appeared to contain larger amounts of antigen than lymphocytes of the bursa of Fabricius (Fig. 49) or thymus (Fig. 50) 4 and 5 DPI.

In the liver viral antigens were present mainly in Kupffer cells 3 DPI. By the fourth and fifth DPI viral antigens were seen in hepatocytes and bile duct epithelium. Foci of hepatic necrosis contained large amounts of viral antigens (Fig. 51). Intranuclear inclusion bodies in hepatocytes also contained large amounts of viral antigen and fluoresced in FASTS preparations (Fig. 52).

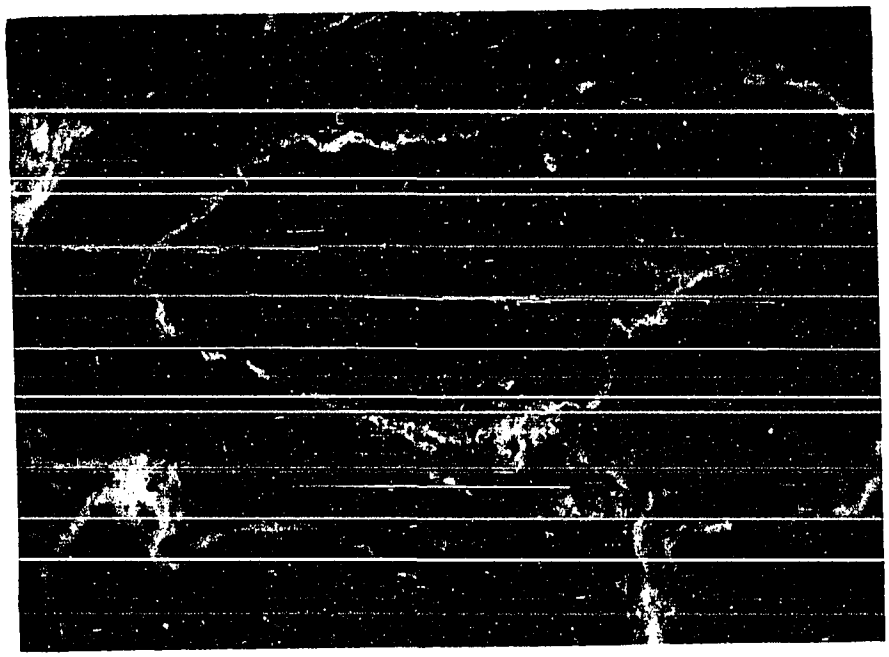
Trial II

Electron microscopy

Esophagus and cloaca Cytoplasmic vacuoles with and without virions and swollen mitochondria with broken cristae were present in the stratum spinosum 3 DPI. Nucleocapsids and fine granular material were present in the center of the nuclei, and chromatin was clumped around the nuclear membranes (Fig. 53). Swollen mitochondria and focal dilation of the endoplasmic reticulum were observed in esophageal glandular epithelium, fibrocytes, macrophages, and lymphocytes in the submucosa. By the fourth and fifth DPI, focal cytolysis of

Figure 49. Bursa of Fabricius 4 DPI, Trial I. Large amounts of viral antigen are present in the epithelial cells separating the cortex and medulla of the bursal follicle. Scattered cells in the cortex and medulla also contain viral antigen. FASTS. x150

Figure 50. Thymic medulla 4 DPI, Trial I. Large amounts of viral antigen are present in cells of Hassel's corpuscle. Smaller amounts of viral antigen are present in cells between the thymic corpuscles. FASTS. x300



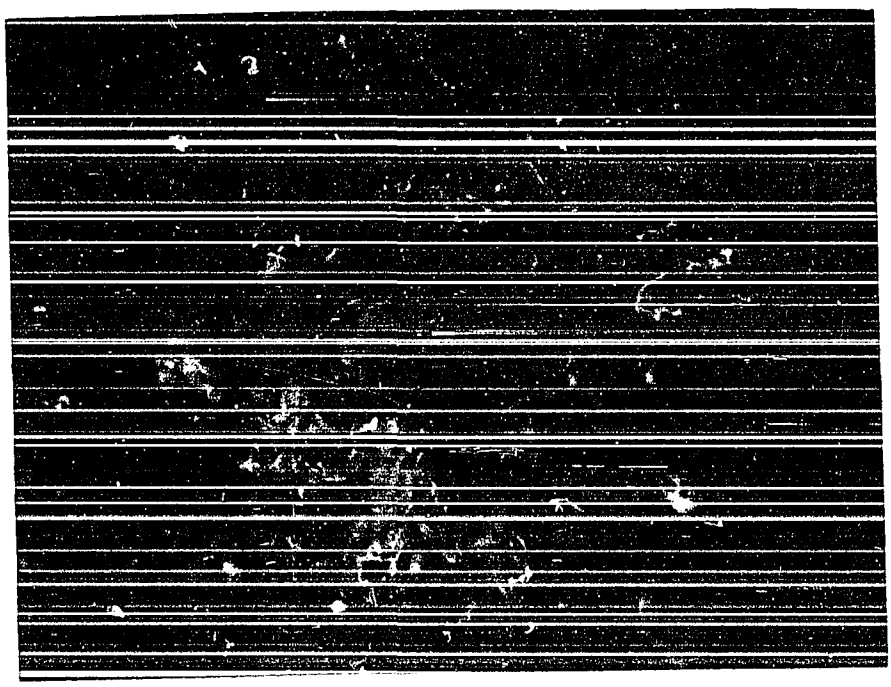
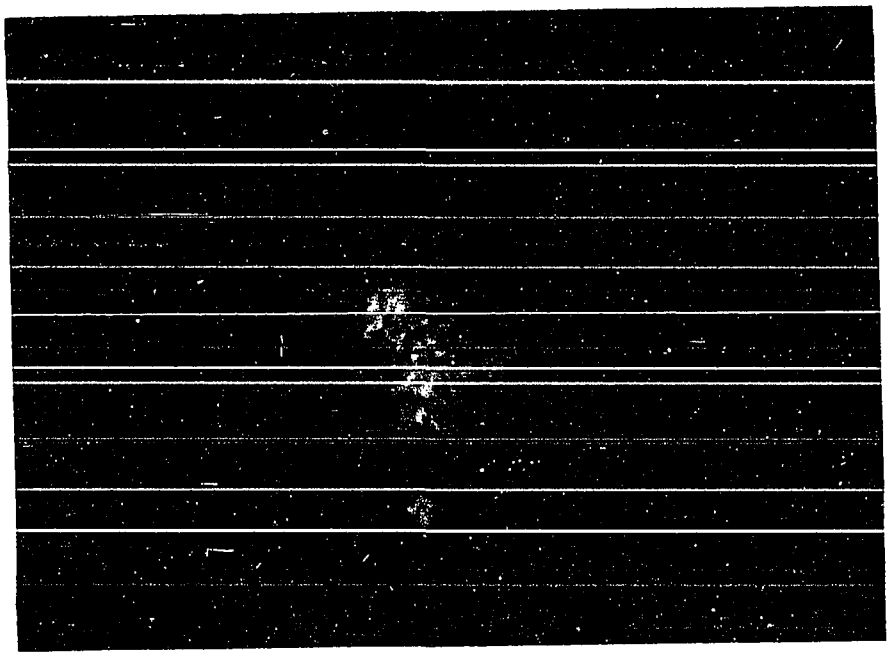


Figure 51. Liver 4 DPI, Trial I. The focus of hepatic necrosis near the center contains large amounts of viral antigen. FASTS. x300

Figure 52. Liver 4 DPI, Trial I. The spherical foci are intranuclear inclusion bodies in hepatocytes. Viral antigen is present in the cytoplasm of hepatocytes. FASTS. x450



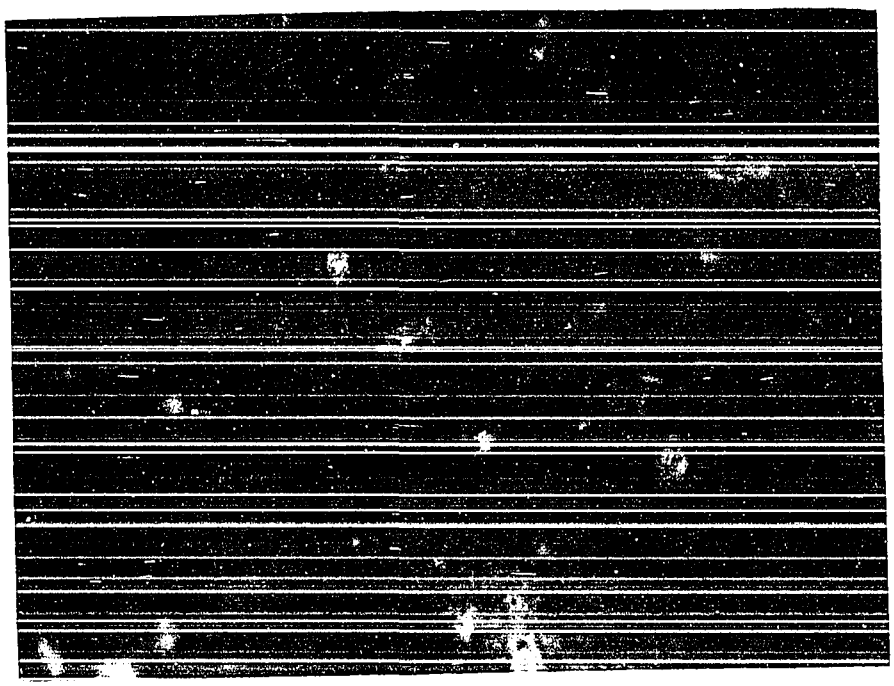
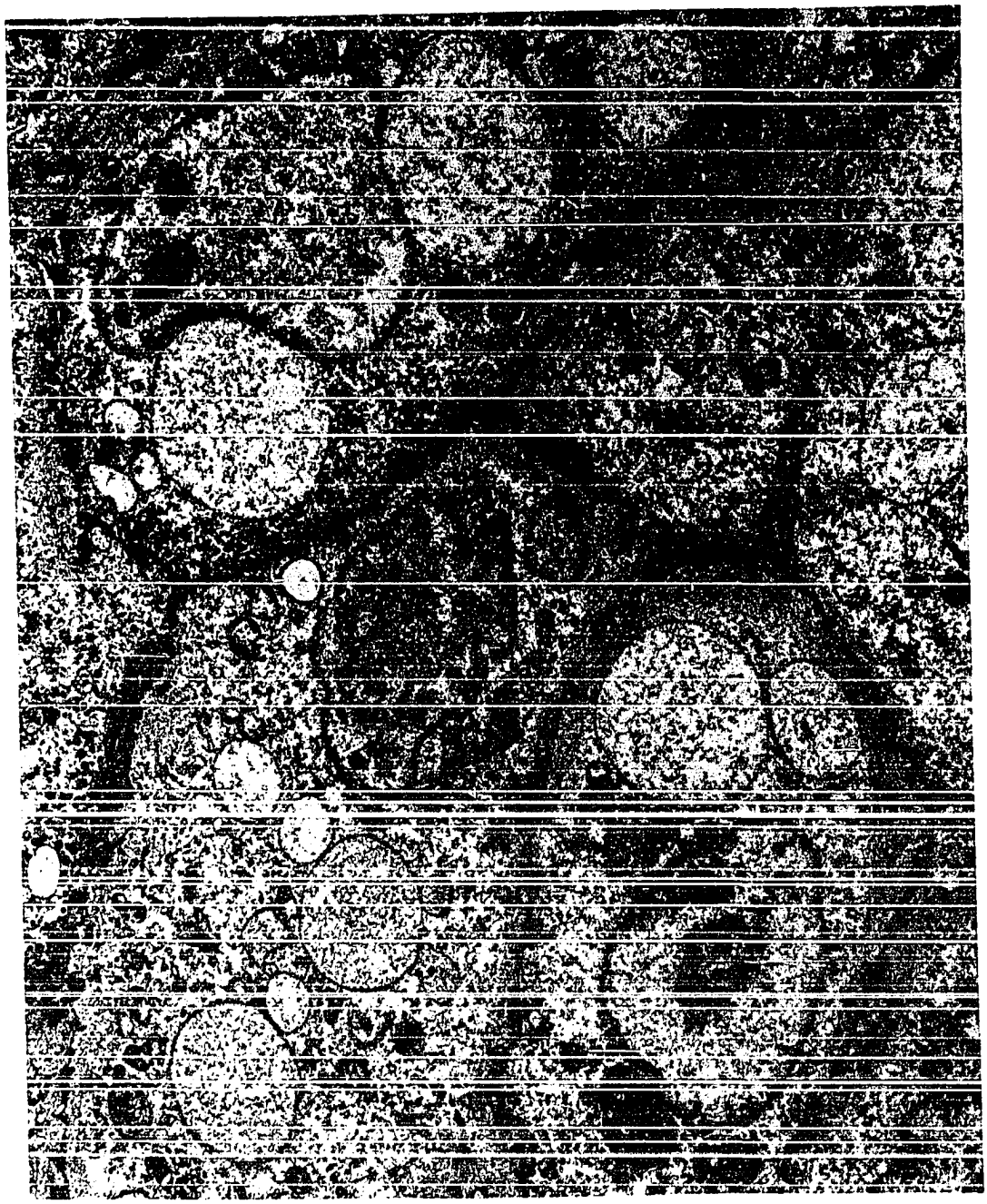


Figure 53. Esophageal mucosa 3 DPI, Trial II. Intracellular edema, cytoplasmic vacuolation, swollen and ruptured mitochondria, clumped and marginated chromatin, cytoplasmic vacuoles with virions, and nucleocapsids in the karyoplasm are present in the epithelial cells. x6,300



epithelial cells in the stratum spinosum was evident. The small vesicles contained cellular detritus and virions (Figs. 54 and 55). In the submucosa, fibrocytes, macrophages, and glandular epithelium were degenerate. Chromatin margination, irregular invaginations of the nuclear membranes, nucleocapsids in the karyoplasm, and nucleocapsids partially budded through the inner nuclear membrane were observed in fibrocytes (Figs. 56, 57, and 58), macrophages, and esophageal glandular epithelium (Figs. 59, 60, and 61). Swollen mitochondria, intracellular edema, virions in vacuoles, and cytolysis were observed in the cytoplasm of these cells. Ultrastructural changes in the lymphocytes will be described in the section on intestine.

Small and large intestines Three DPI chromatin margination and phagolysosomes were seen in macrophages and fibrocytes of the villous lamina propria. Focal dilation of the endoplasmic reticulum and marginated chromatin were present in crypt epithelium. Four and 5 DPI clumped chromatin, irregular dilation of perinuclear cisternae, vacuolation of mitochondria, cytolysis, and karyorrhexis were observed in lymphocytes of Meckel's diverticulum and annular bands. Nucleocapsids were found in 9% of 200 lymphocytic nuclei examined (Fig. 62). Five of these lymphocytes also had nucleocapsids in the cytoplasm around dense bodies (Fig. 63).

Figure 54. Cloacal mucosa 5 DPI, Trial II. An occasional heterophil is present among the lysed epithelial cells. Scattered pockets of virions are present among the cellular detritus. x2,500

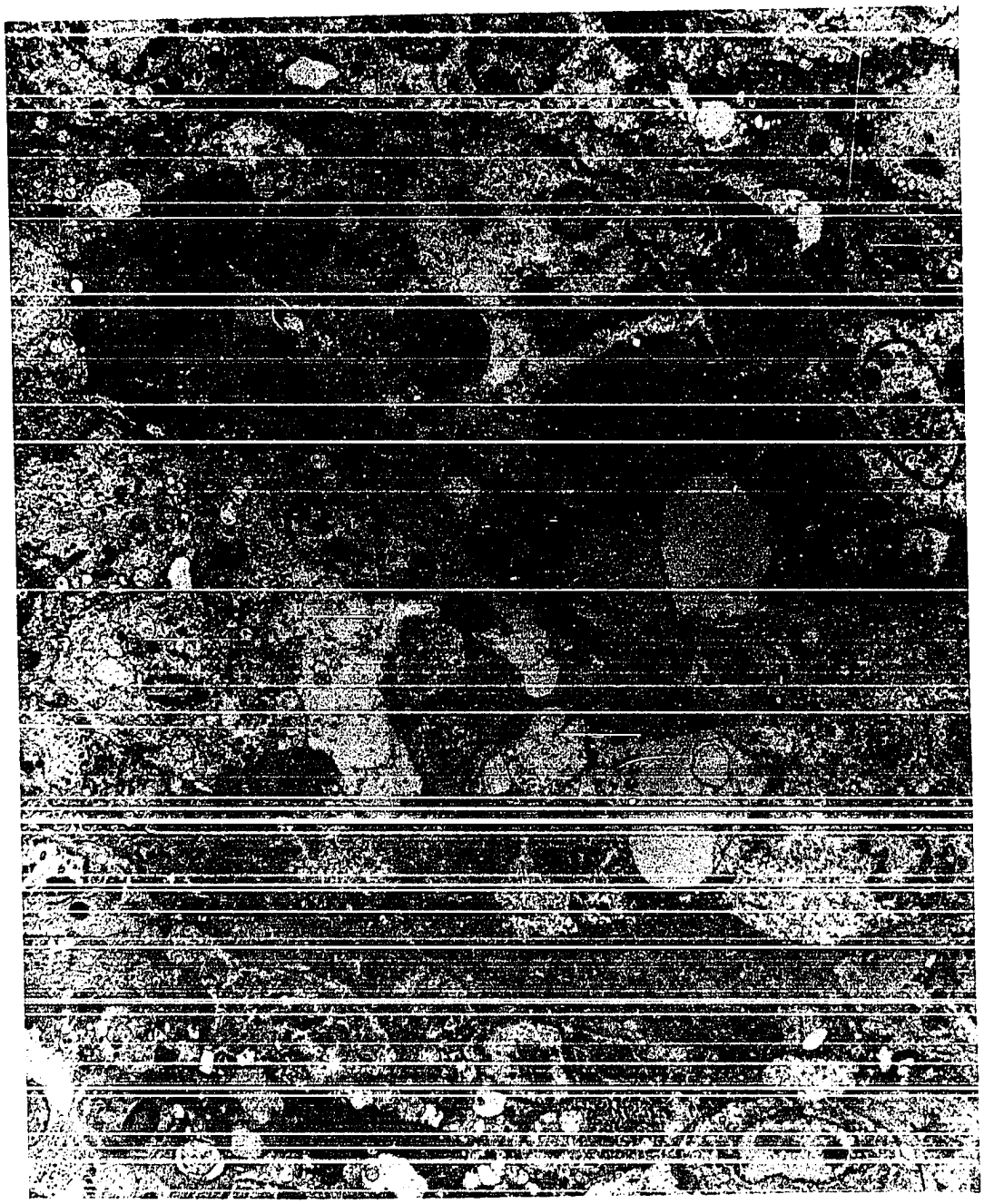


Figure 55. Cloacal mucosa 5 DPI, Trial II. Several small vesicles containing cellular detritus and virions are present in the stratum spinosum. Most organelles in the lysed epithelial cells are missing. x4,800

Figure 56. Submucosa of esophagus 4 DPI, Trial II. Chromatin in fibrocytes is clumped and marginated, and vacuoles are present in the cytoplasm. A partially degranulated mast cell and a small artery with swollen endothelium are also present. x4,200

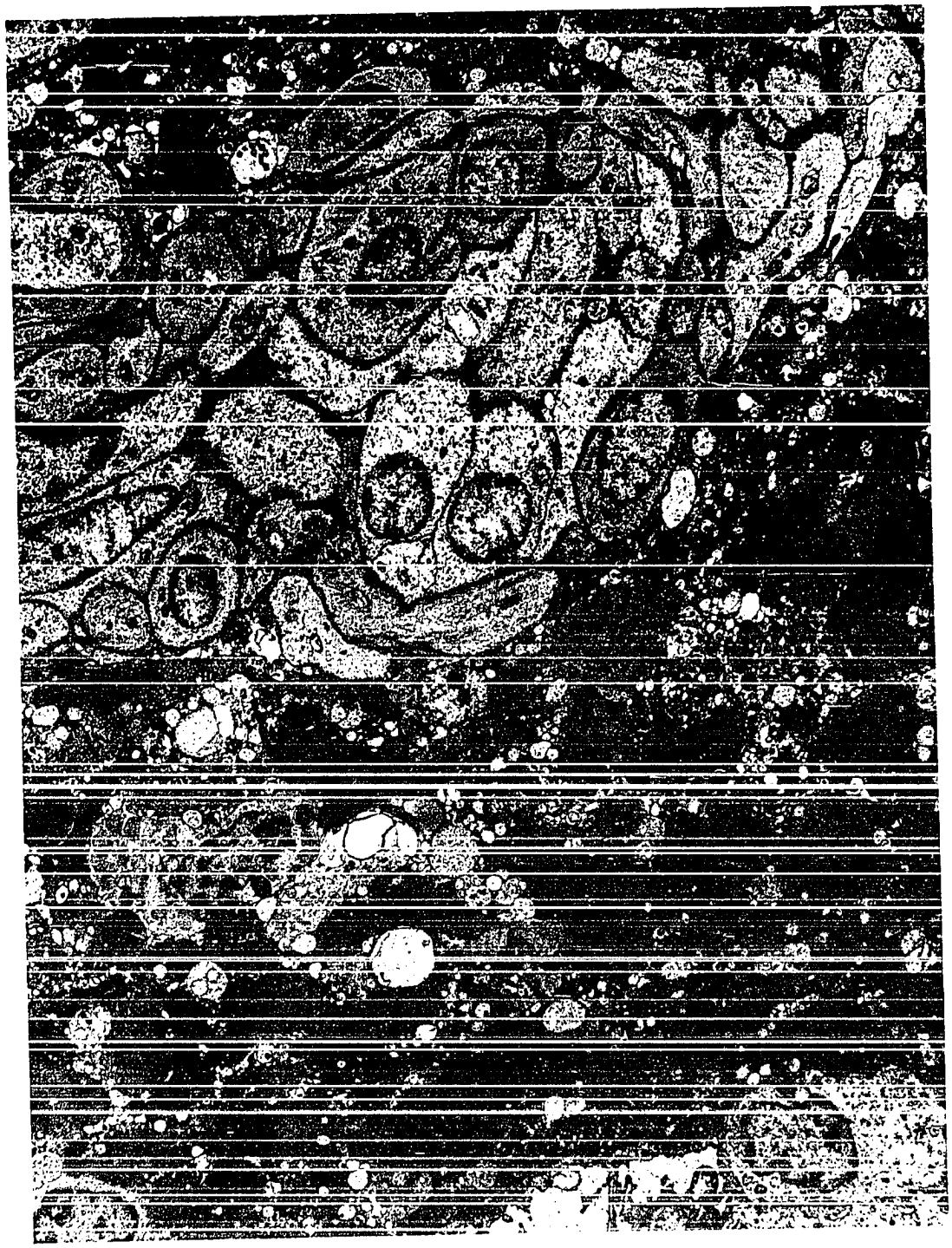


Figure 57. Esophageal submucosal fibrocyte 4 DPI, Trial II. The chromatin is clumped and marginated, and nucleocapsids are present in the nucleus. Irregular folds are present in the nuclear membrane, and the perinuclear cisterna is dilated. Mitochondria are swollen, and most of the cristae are missing. x32,500

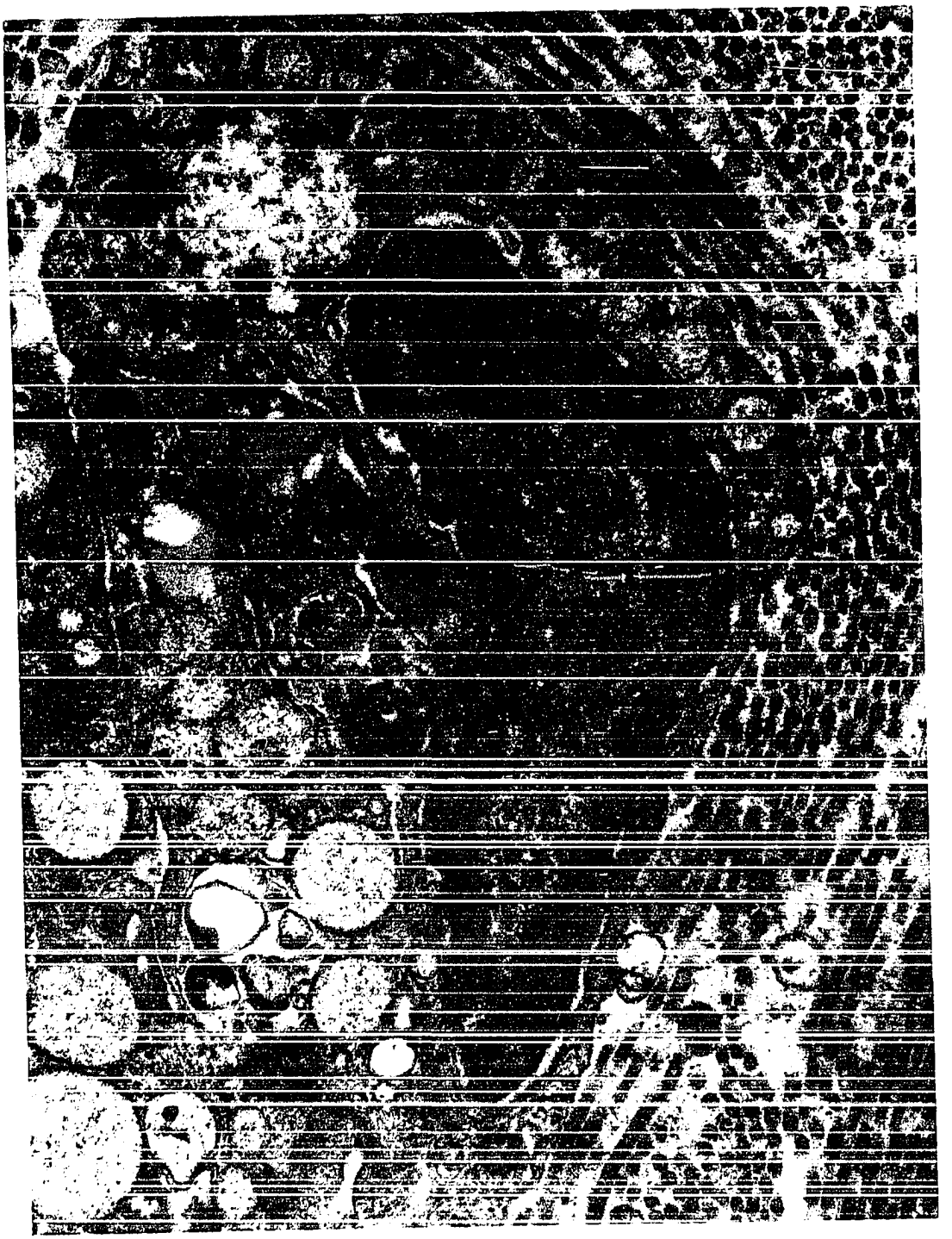


Figure 58. Esophageal submucosal fibrocyte 5 DPI, Trial II. A nucleocapsid is seen partially budded through the inner nuclear membrane. An autophagic vacuole and focal dilation of the endoplasmic reticulum are present in the cytoplasm, and nucleocapsids are present in the nucleus. x38,400

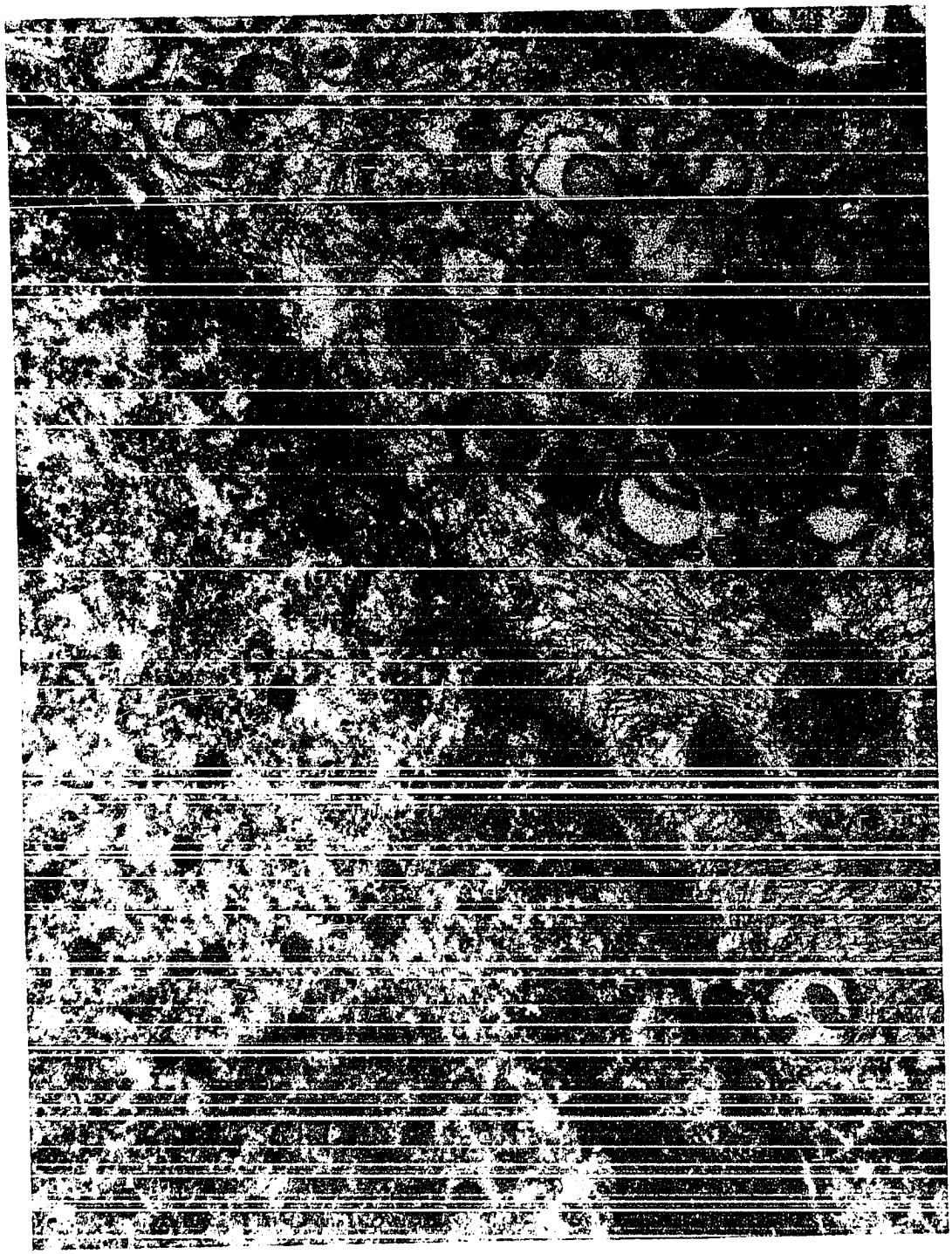


Figure 59. Esophageal submucosal gland 4 DPI, Trial II. Vacuoles which contain mucus and virions, swollen mitochondria, dilated endoplasmic reticulum, and edema are present in the cytoplasm. The chromatin is clumped and marginated. Fibrocytes beneath the basement membrane are degenerate, and the chromatin is clumped and marginated. x4,200

Figure 60. Submucosal esophageal gland 4 DPI, Trial II. Virions are present in cytoplasmic vacuoles and in between cells. Intracellular edema, focal dilation of the endoplasmic reticulum, chromatin margination, and nucleocapsids are present in the epithelial cells. x17,500

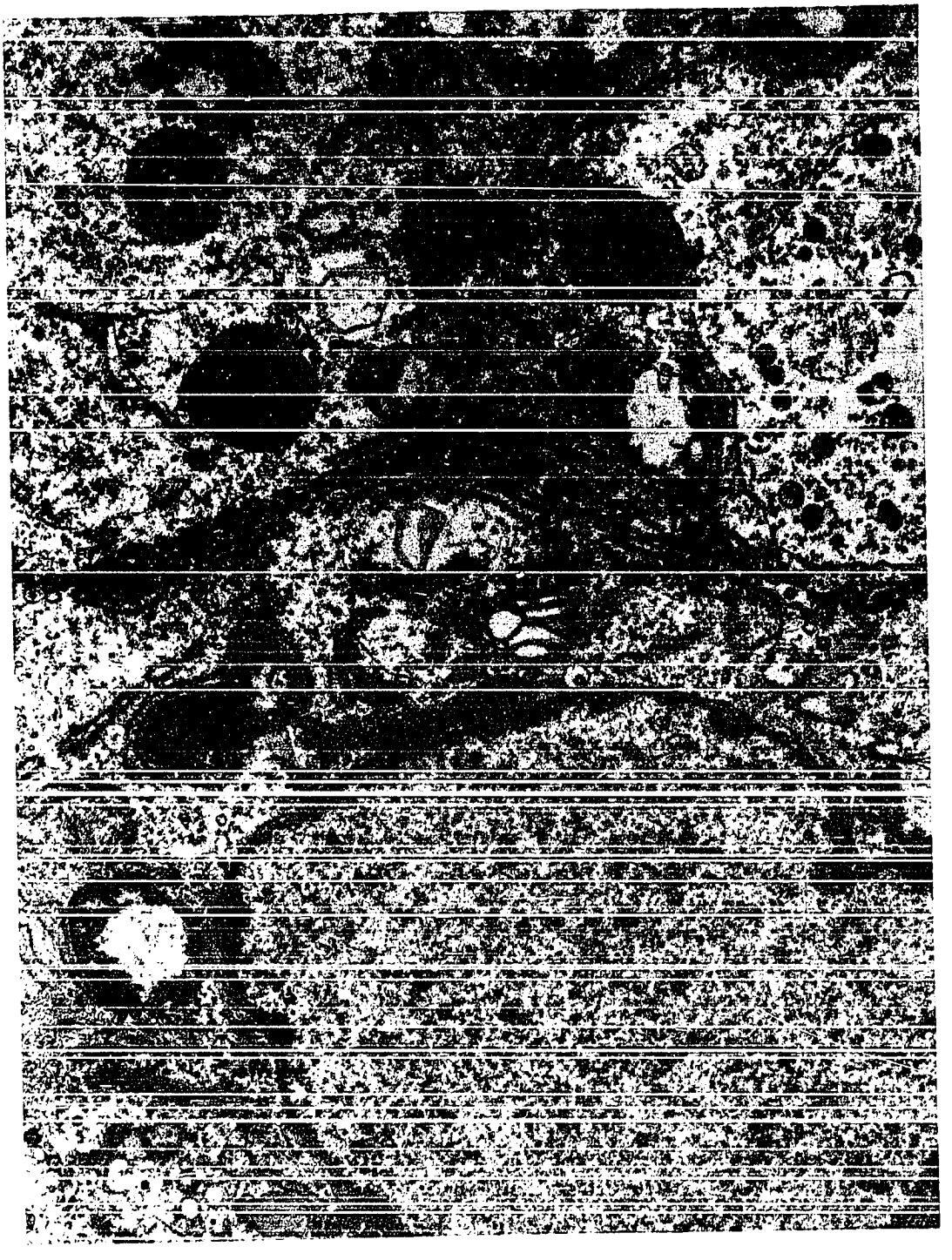


Figure 61. Submucosal esophageal gland 4 DPI, Trial II. Focal segmental reduplications of the inner nuclear membrane are present in the irregularly dilated perinuclear cisterna. x45,500

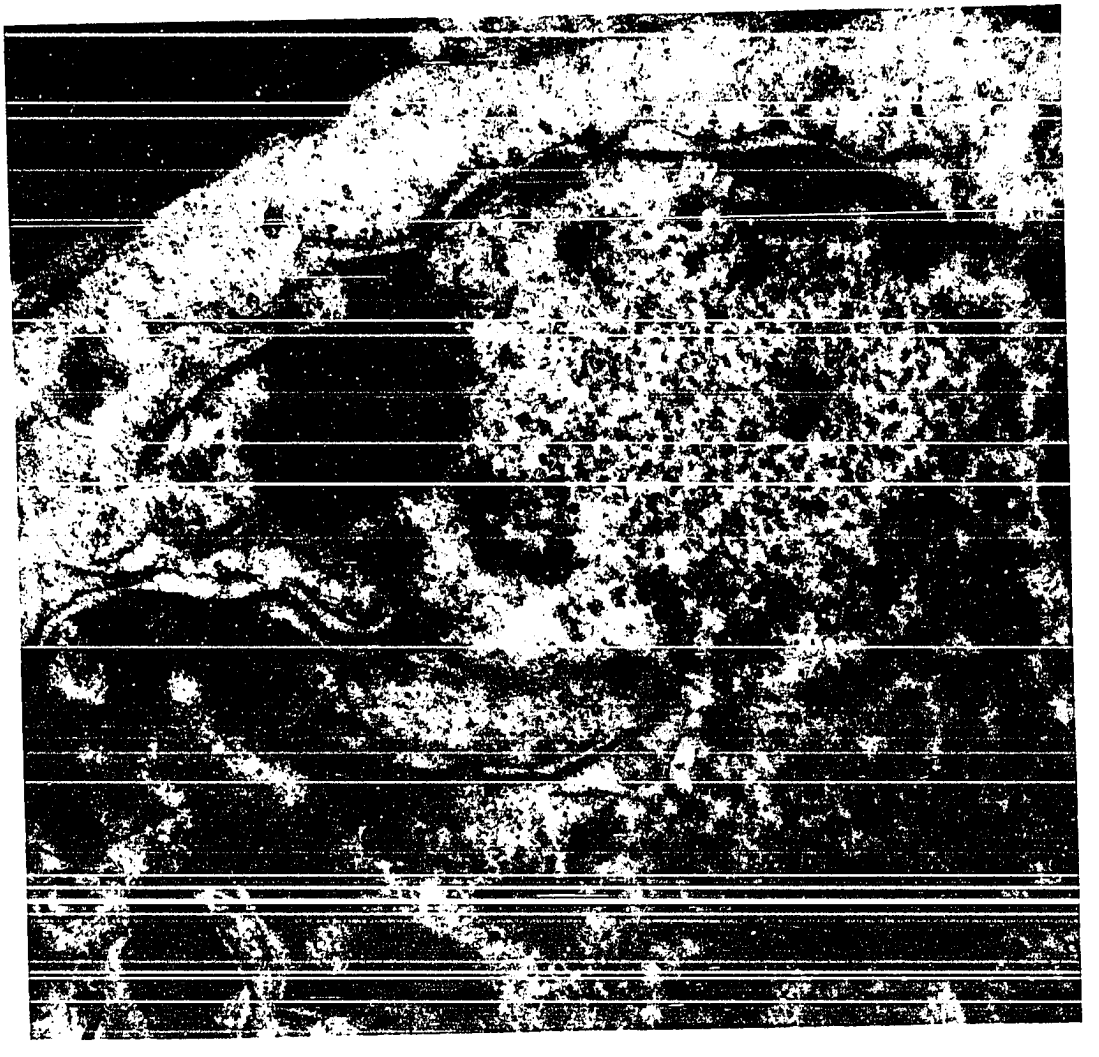


Figure 62. Annular band 4 DPI, Trial II. Lymphocytic degeneration is characterized by clumped chromatin, swollen mitochondria, dilation of the perinuclear cisternae, and cytolysis. A virion is present in a lymphocyte in the upper righthand corner. x13,200

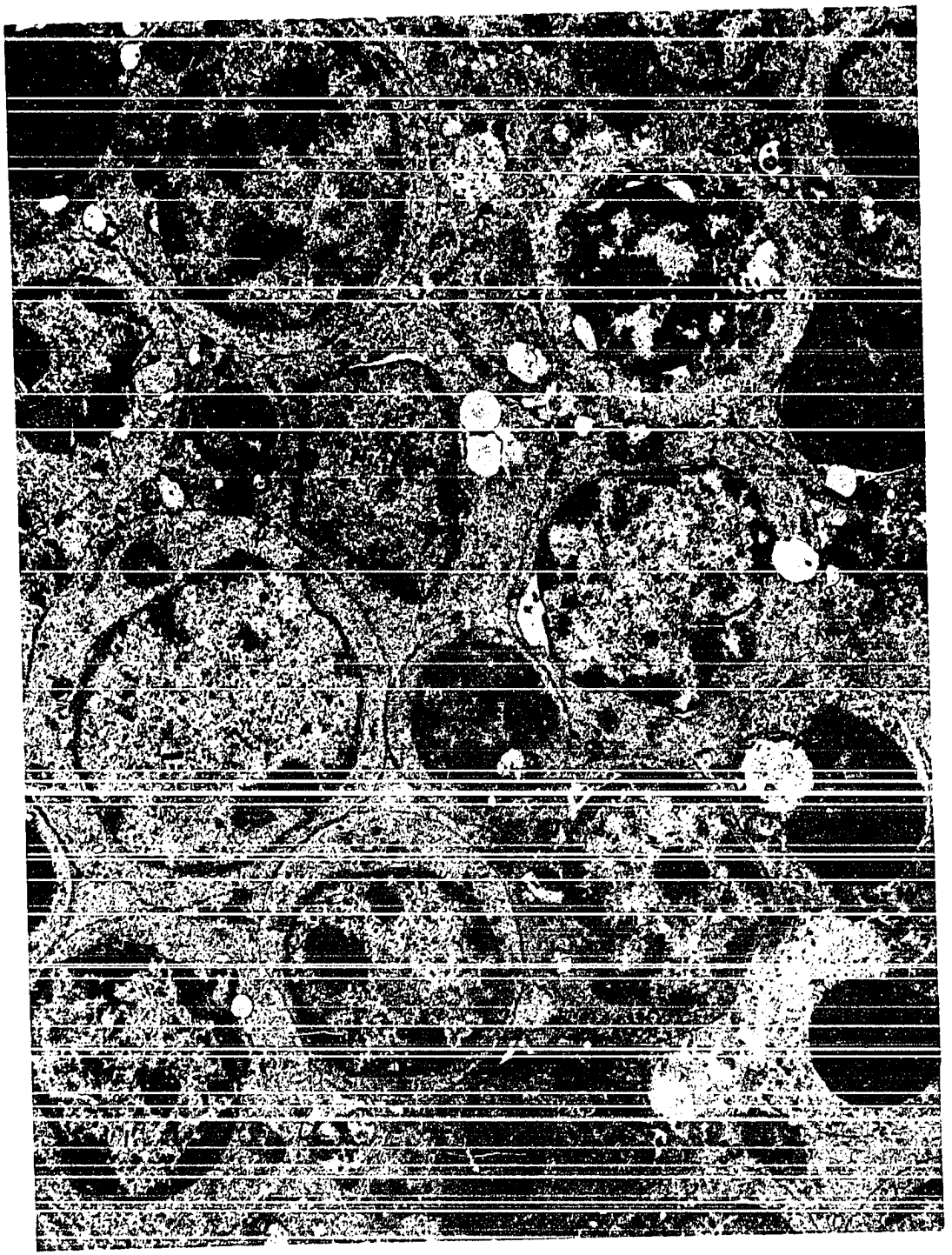


Figure 63. Annular band lymphocyte 4 DPI, Trial II. Nucleocapsids are present in the nucleus and around a dense body in the cytoplasm. x30,000

Enveloped virions were found in the cytoplasm of one lymphocyte (Fig. 62). Histiocytes and heterophils in the villous lamina propria contained nucleocapsids (Figs. 64 and 65) in the nuclei and virions in the cytoplasm. Viral particles were not found in villous epithelium, but virions and nucleocapsids were seen in crypt epithelium (Figs. 66 and 67). Degeneration and necrosis of these cells occurred after viral maturation.

Spleen Three DPI swollen mitochondria and focal dilation of the endoplasmic reticulum were present in lymphocytes and reticuloendothelial cells. By 4 DPI cytolysis had occurred in many lymphocytes of germinal centers and periarteriolar reticular sheaths. Chromatin was clumped in the nuclei of the remaining lymphocytes, and nucleocapsids were present in the nuclei (Figs. 68 and 69). Virions were infrequently found in lymphocytes. Nucleocapsids and virions were present in reticuloendothelial cells of the periarteriolar reticular sheath, and in macrophages lining splenic sinusoids. Cytoplasmic edema, swollen mitochondria, phagolysosomes, chromatin margination, and cytolysis were also present in these cells (Figs. 70, 71, and 72). By the fifth DPI most of the reticuloendothelial cells and lymphocytes were necrotic.

Bursa of Fabricius Three DPI dilation of the perinuclear cisternae and cytolysis were seen in a few medullary lymphocytes. Swollen mitochondria with broken cristae,

Figure 64. Heterophil in the submucosa of the small intestine 4 DPI, Trial II. Chromatin is clumped and marginated, and a nucleocapsid is present in the nucleus. x26,000

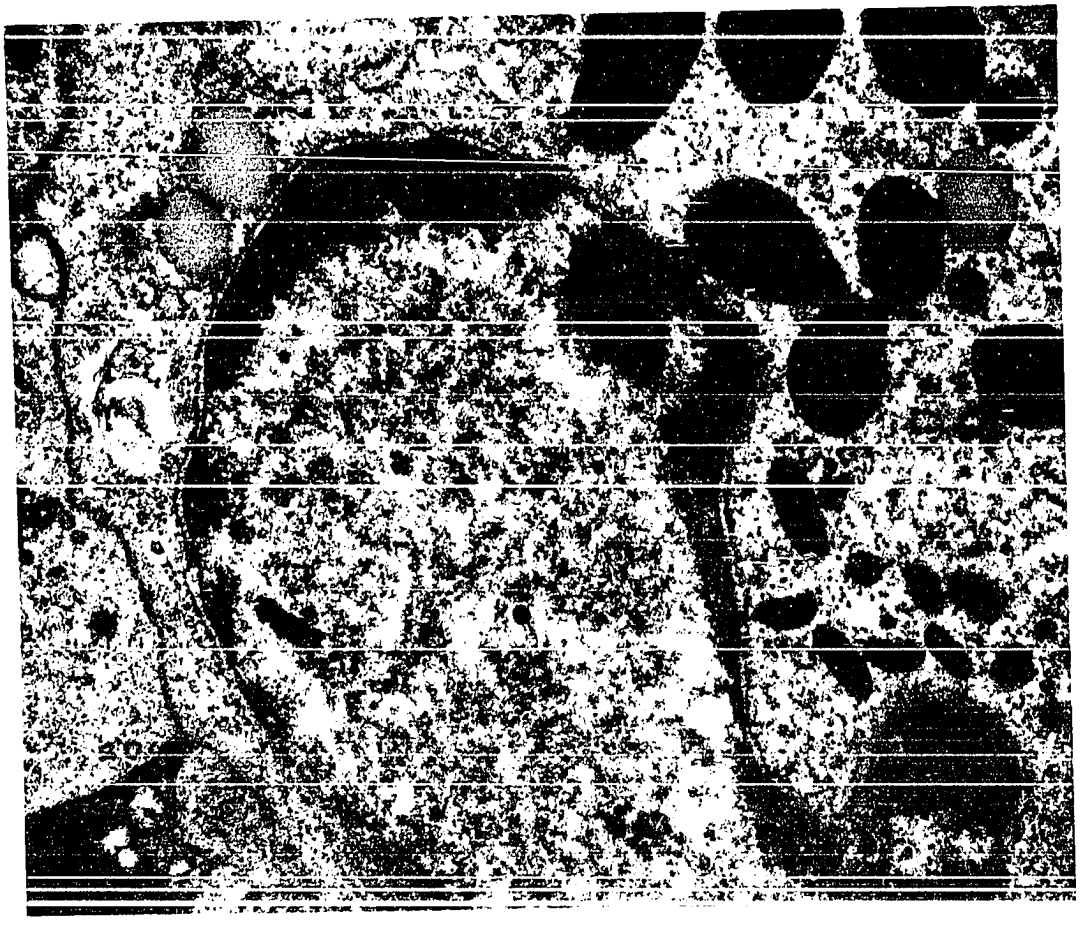


Figure 65. Macrophage in the lamina propria of the large intestine 4 DPI, Trial II. Phagocytized debris is present in the cytoplasm. Chromatin is clumped and marginated, and nucleocapsids are present in the nucleus. x14,700

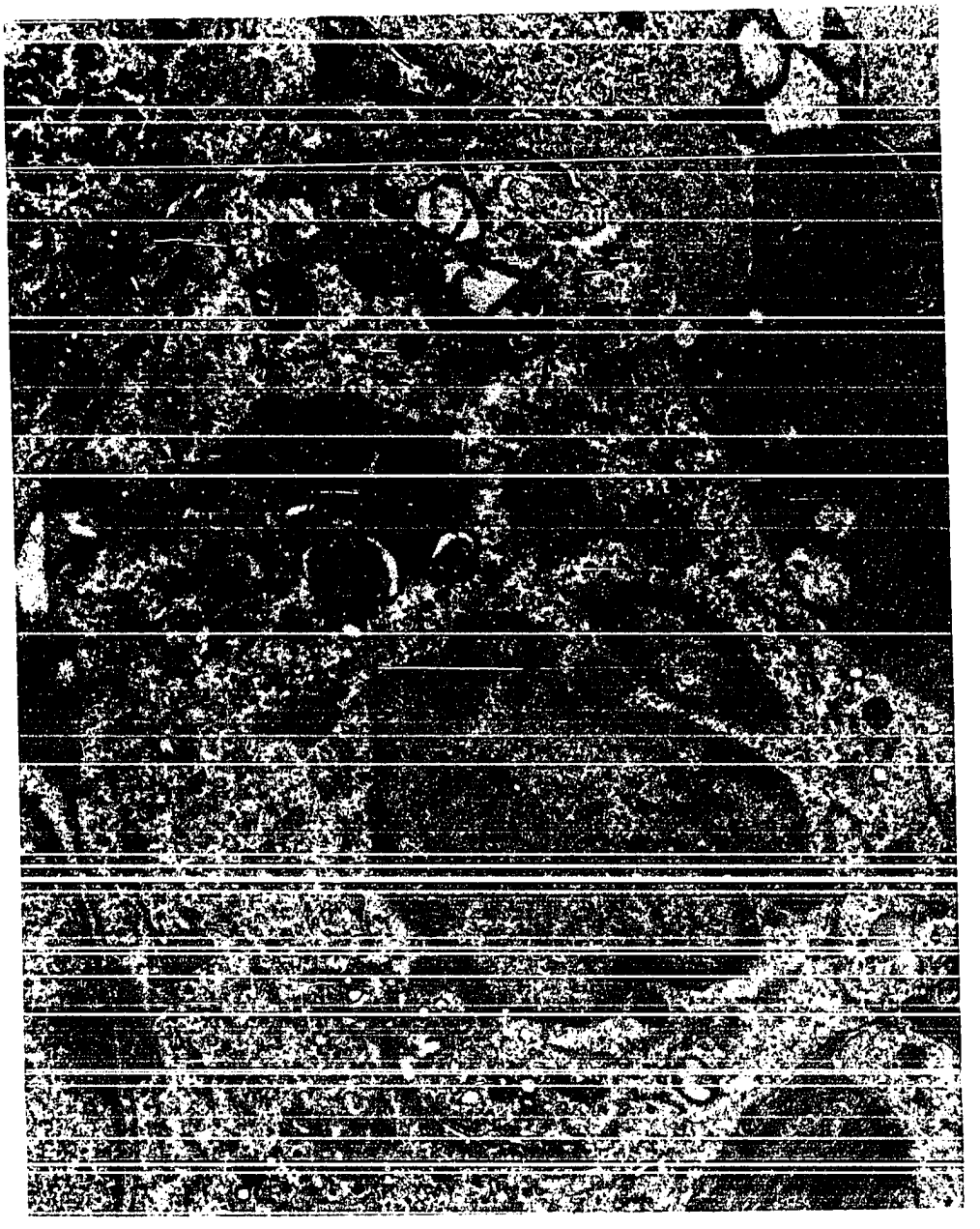


Figure 66. Crypt of Leiberkühn in the ileum 4 DPI, Trial II. Chromatin is clumped and marginated in the epithelial cells. Swollen mitochondria and focal dilation of the endoplasmic reticulum are present in the cytoplasm. x5,000

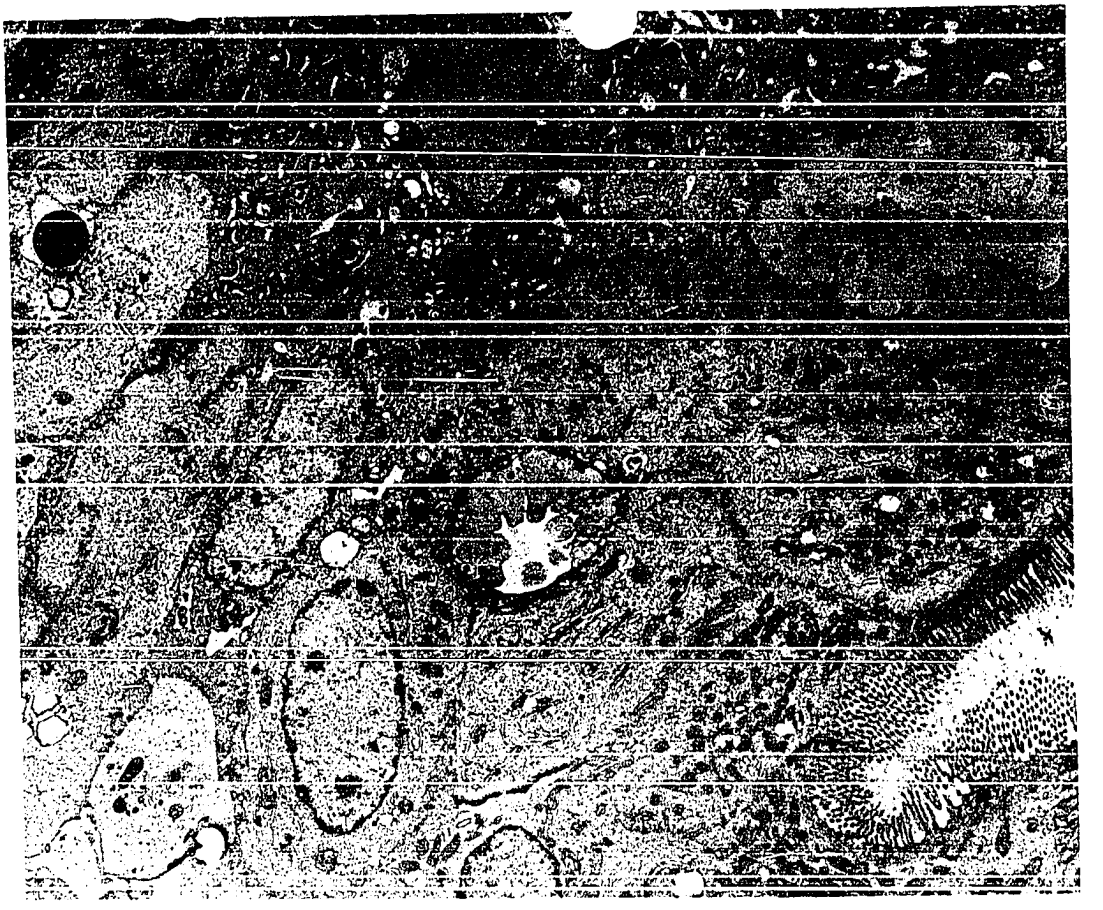


Figure 67. Higher magnification of a nucleus from Fig. 66 containing nucleocapsids. x31,200

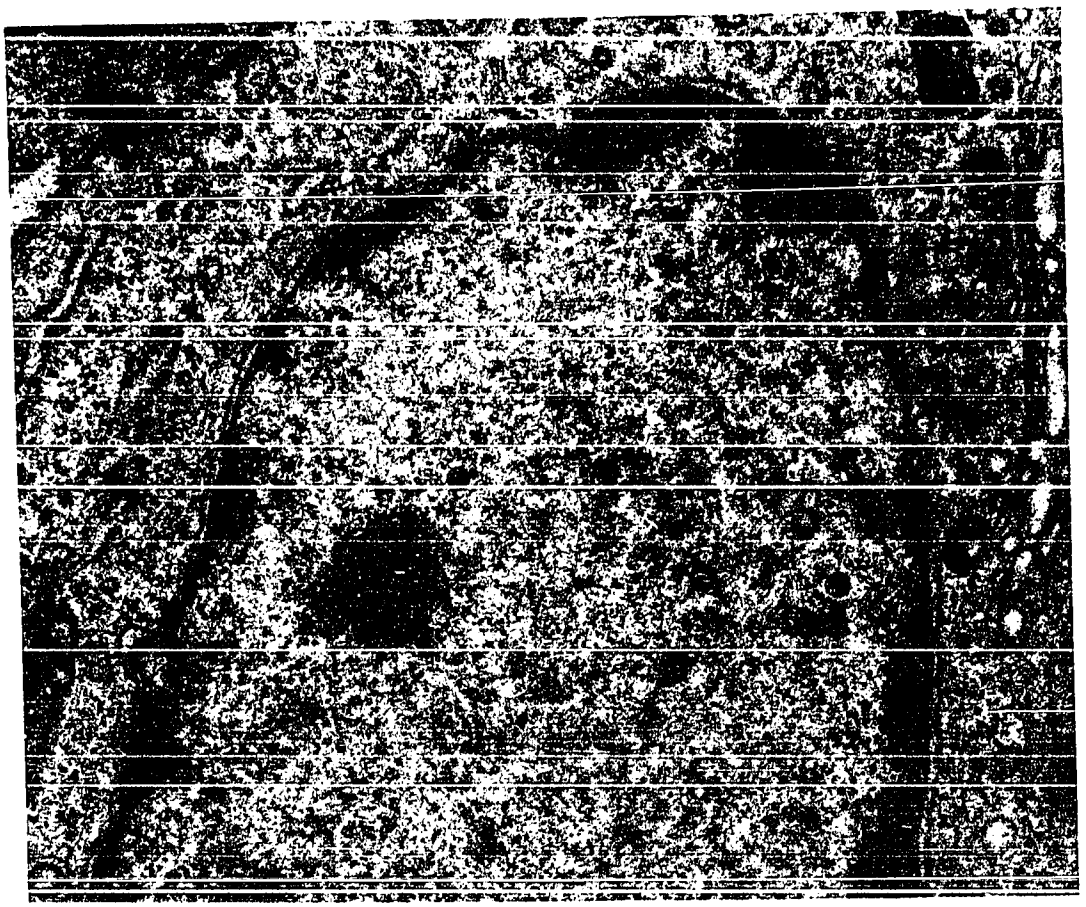


Figure 68. Splenic lymphocyte from periarteriolar lymphoid sheath 4 DPI, Trial II. Mitochondria are swollen, and breaks are present in the surrounding membranes. Cristae are broken, and electron dense material is present in the matrix of the mitochondria. Nucleocapsids are present in the karyoplasm. x19,000

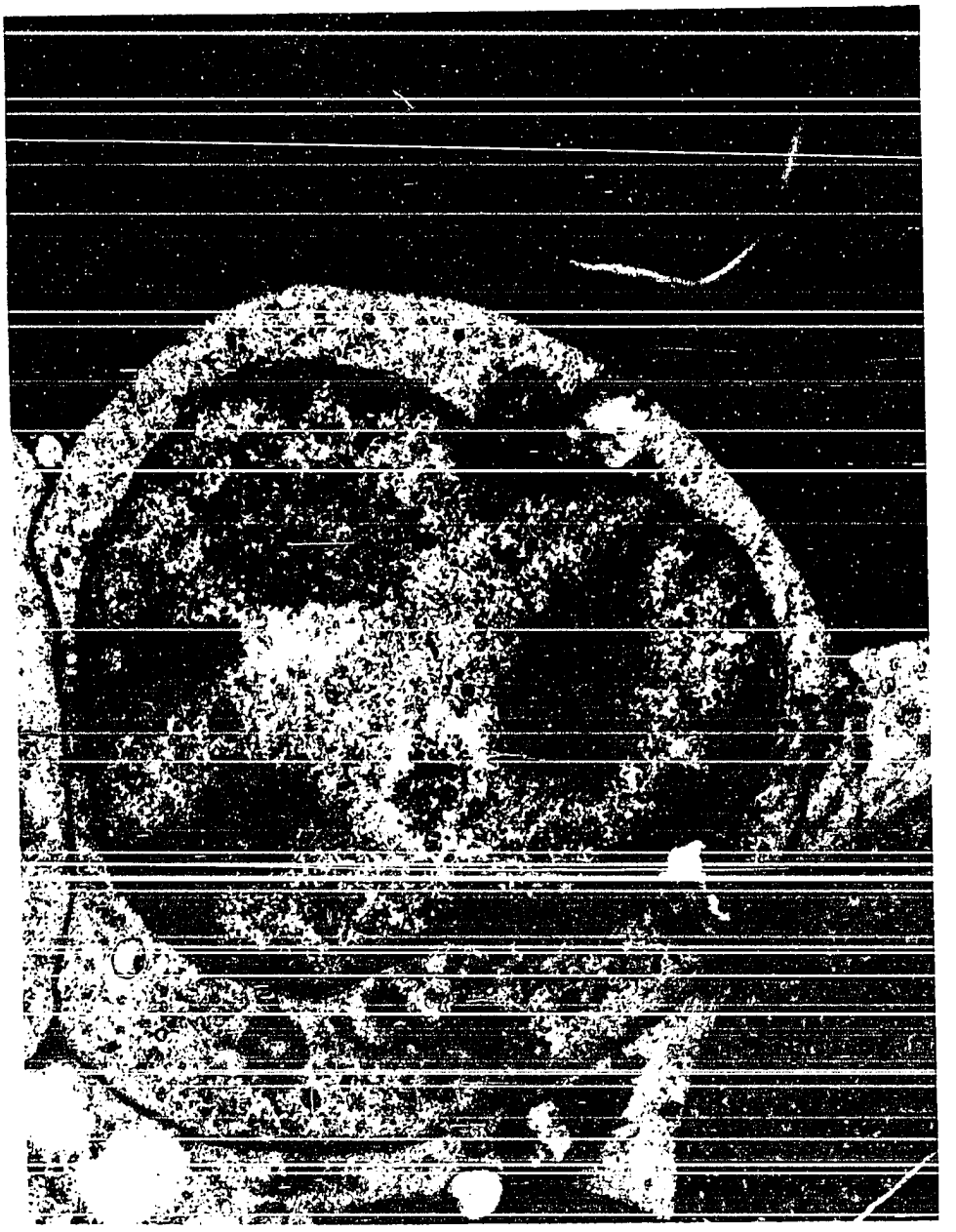


Figure 69. Germinal center lymphocytes from the spleen 4 DPI, Trial II. Necrotic lymphocytes have been phagocytized by a splenic macrophage. Mitochondria in the surrounding lymphocytes are swollen. x8,600

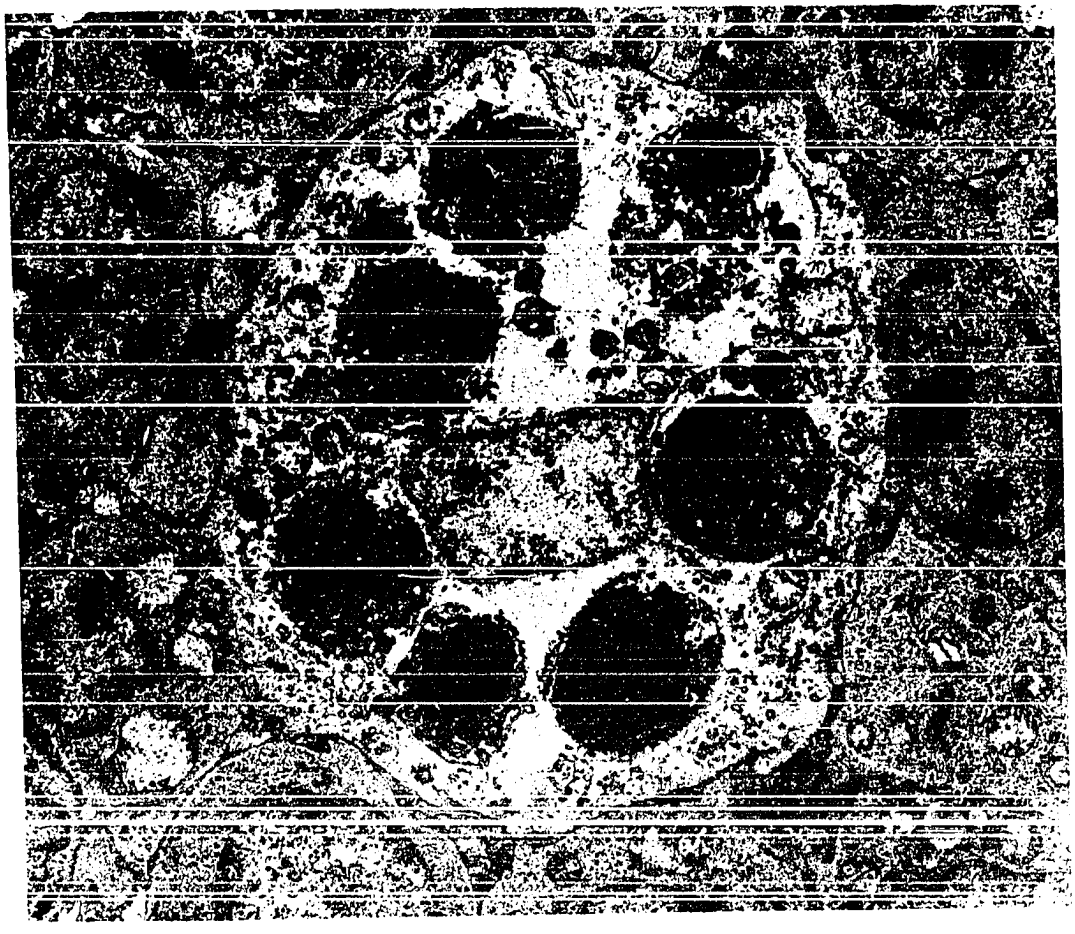


Figure 70. Macrophages in the periarteriolar reticular sheath are degenerate 4 DPI, Trial II. Focal dilation of the endoplasmic reticulum, cytoplasmic edema, and clumped, marginated chromatin are evident in the endothelial cell lining the arteriole near the top of the photograph. x6,800

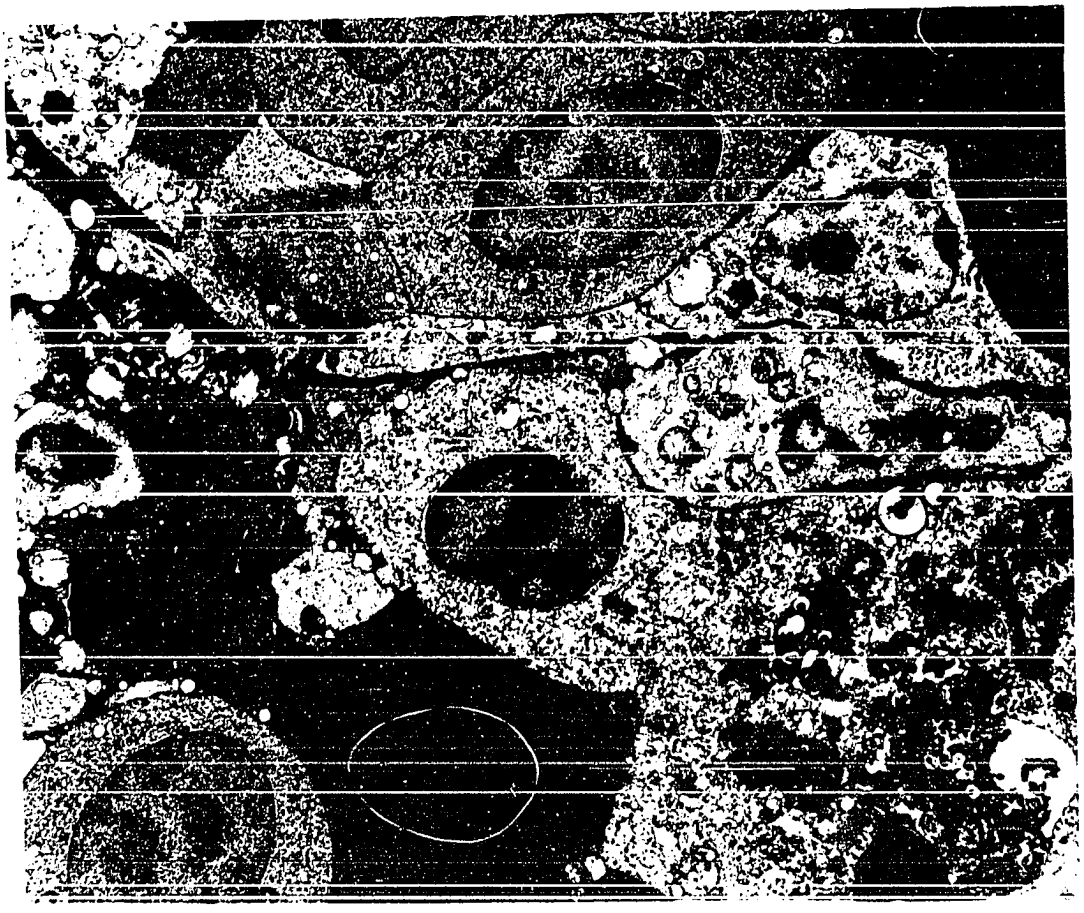


Figure 71. Splenic sinusoidal macrophage 4 DPI, Trial II. The chromatin is clumped and marginated. Nucleocapsids, primary bodies, and fine granular material are present in the karyoplasm. Irregular folds are present in the nuclear membranes, and irregular dilations are present in the perinuclear cisterna. Mitochondria are swollen and contain an electron dense material, and vacuoles are present in the cytoplasm. x14,000

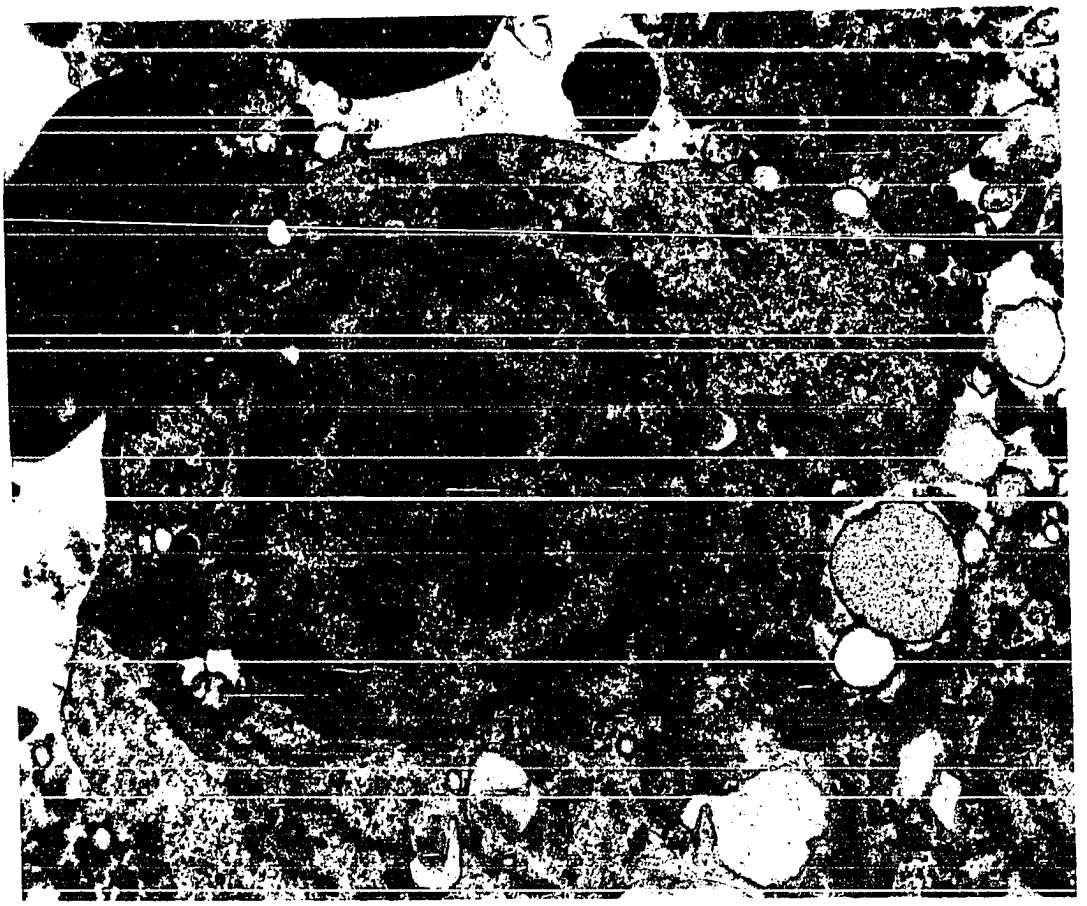


Figure 72. Splenic macrophage 4 DPI, Trial II, with large phagolysosomes, and vacuoles containing virions in the cytoplasm. Chromatin in the nucleus is clumped. x16,000

phagocytized necrotic lymphocytes, clumped chromatin, and nucleocapsids were present in bursal macrophages (Fig. 73). On the fourth and fifth DPI cytolysis was observed in medullary lymphocytes and to a lesser degree in cortical lymphocytes. Very few virions were found in the lymphocytes, but nucleocapsids were frequently found in the nucleus and around dense bodies in the cytoplasm (Fig. 74). Scattered degenerate plasma cells with cytoplasmic vacuoles, swollen mitochondria, marginated chromatin, and focal reduplications of the nuclear membrane were found in the follicles (Fig. 75). Virions were found in cytoplasmic vacuoles of degenerate epithelial cells separating the cortex and medulla, and nucleocapsids were found in the cytoplasm and nuclei. Mitochondria were swollen, and the cristae were broken (Fig. 76). Cytolysis was also observed in interfollicular fibrocytes (Fig. 77).

Thymus Ultrastructural changes were initially seen 4 DPI in the thymus. Virions were found in cytoplasmic vacuoles of squamous, cystic, and villous epithelial cells of Hassel's corpuscles. Focal dilations in the endoplasmic reticulum and cytoplasmic edema were present. Mitochondria were swollen, and cristae were broken. Chromatin was clumped and marginated, and nucleocapsids and fine granular material were present in the center of the nuclei (Figs. 78 and 79). Cytolysis and karyorrhexis had occurred in many medullary lymphocytes.

Figure 73. Macrophage in the bursa of Fabricius 3 DPI, Trial II. Two phagocytized necrotic lymphocytes, swollen mitochondria, marginated chromatin, and nucleocapsids in the karyoplasm are present. x18,200

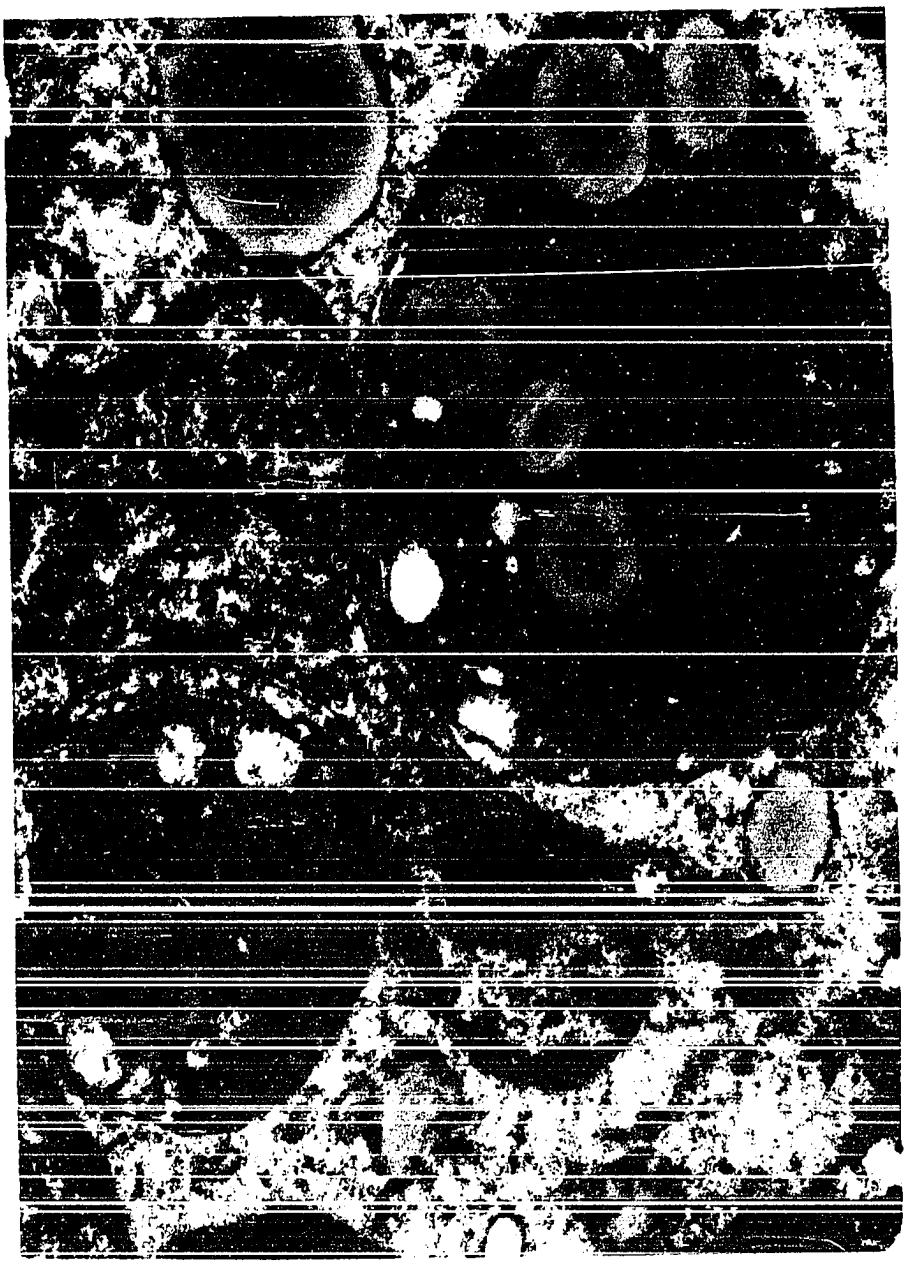


Figure 74. Cortical lymphocytes from a bursa of Fabricius follicle 4 DPI, Trial II. Chromatin is clumped in lymphocytes. Nucleocapsids are present in the cytoplasm near dense bodies of the lymphocyte in the upper left. Chromatin is clumped and marginated in the degenerate macrophage. A few nucleocapsids and a phagocytized lymphocyte are present in the cytoplasm. x15,000

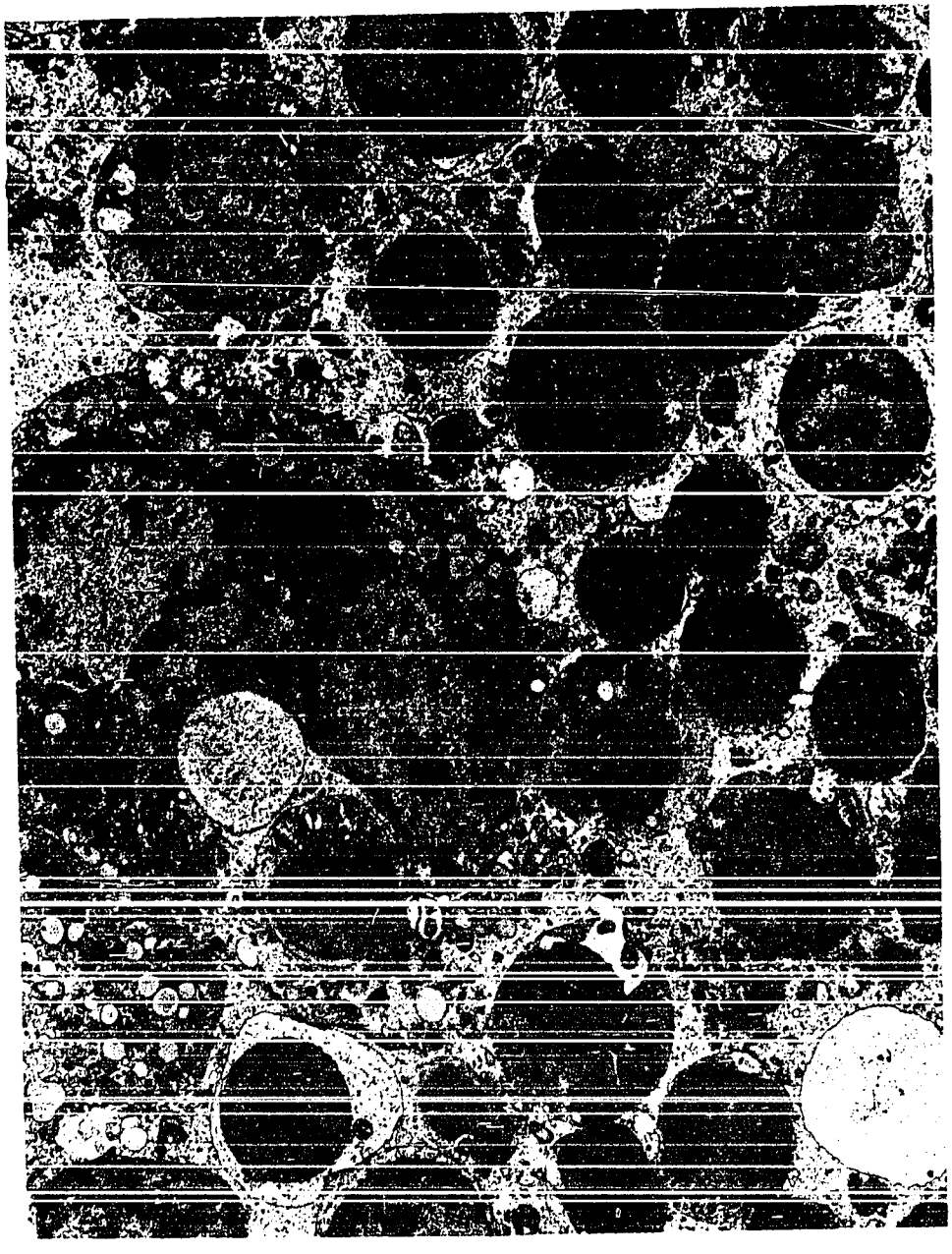


Figure 75. Plasma cell from the bursa of Fabricius 4 DPI, Trial II. Cytolysis and karyorrhexis, cytoplasmic vacuoles, swollen mitochondria, clumped chromatin, and reduplicated inner nuclear membrane are present. x26,000

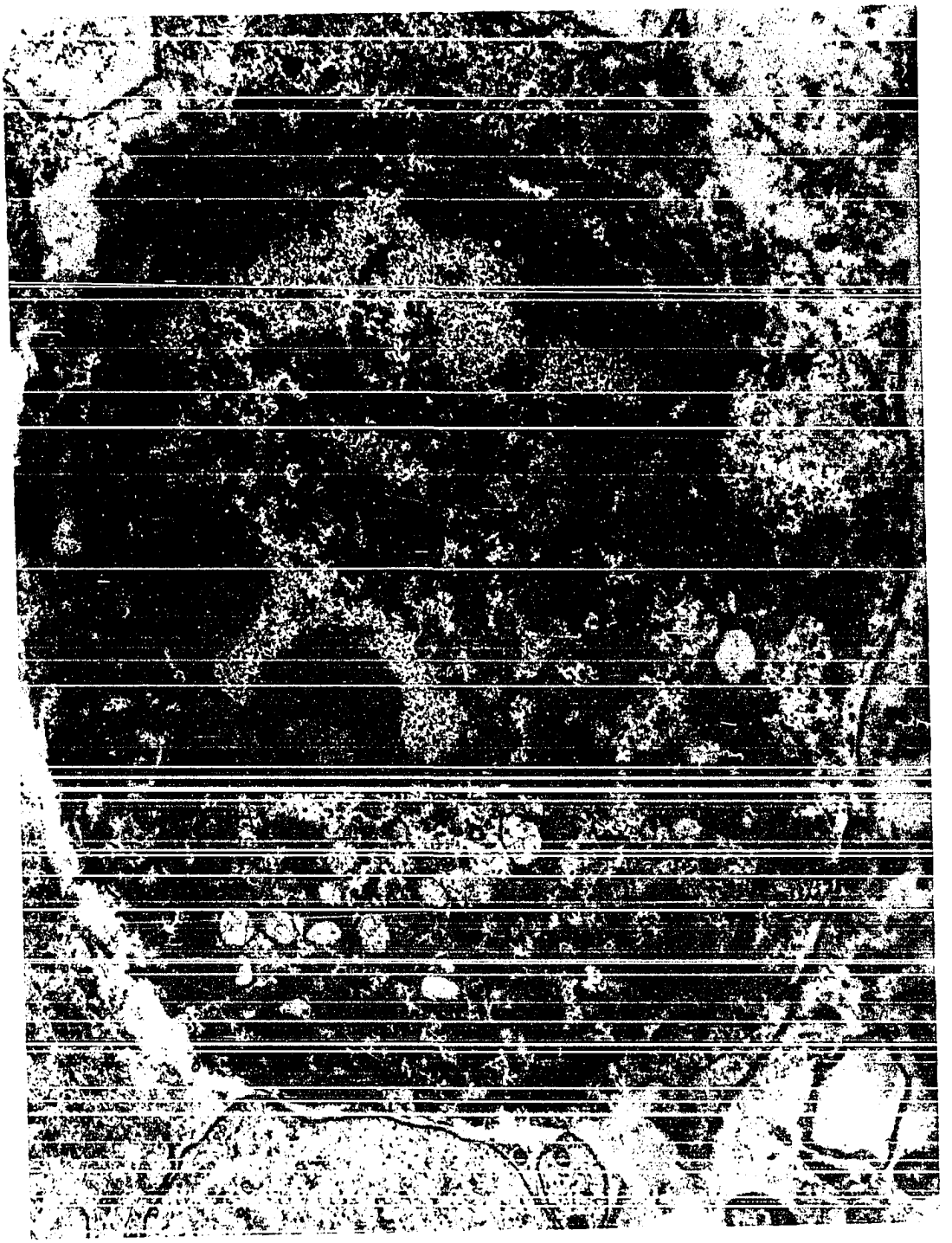


Figure 76. Epithelial cells separating the cortex and medulla of a bursa of Fabricius follicle 4 DPI, Trial II. Tonofilaments are present in the cytoplasm, and desmosomes are present between these cells. Intracellular edema, swollen and ruptured mitochondria with broken cristae, nucleocapsids, and vacuoles containing virions are present in the cytoplasm. x9,500

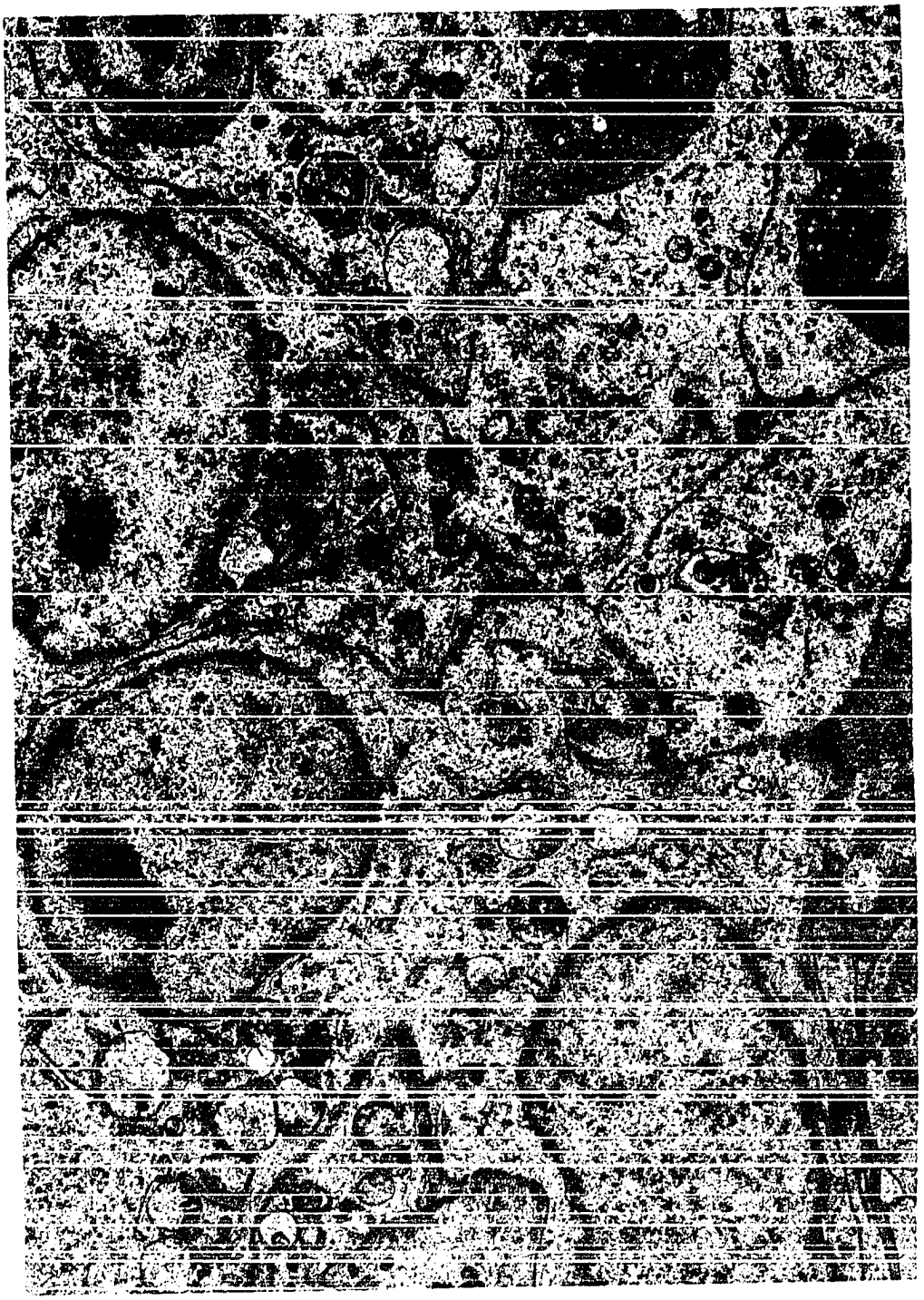


Figure 77. Interfollicular fibrocytes in the bursa of Fabricius 5 DPI, Trial II. Cytolysis and karyolysis have occurred in the fibrocytes. Nucleocapsids and virions are scattered among the detritus. x13,200

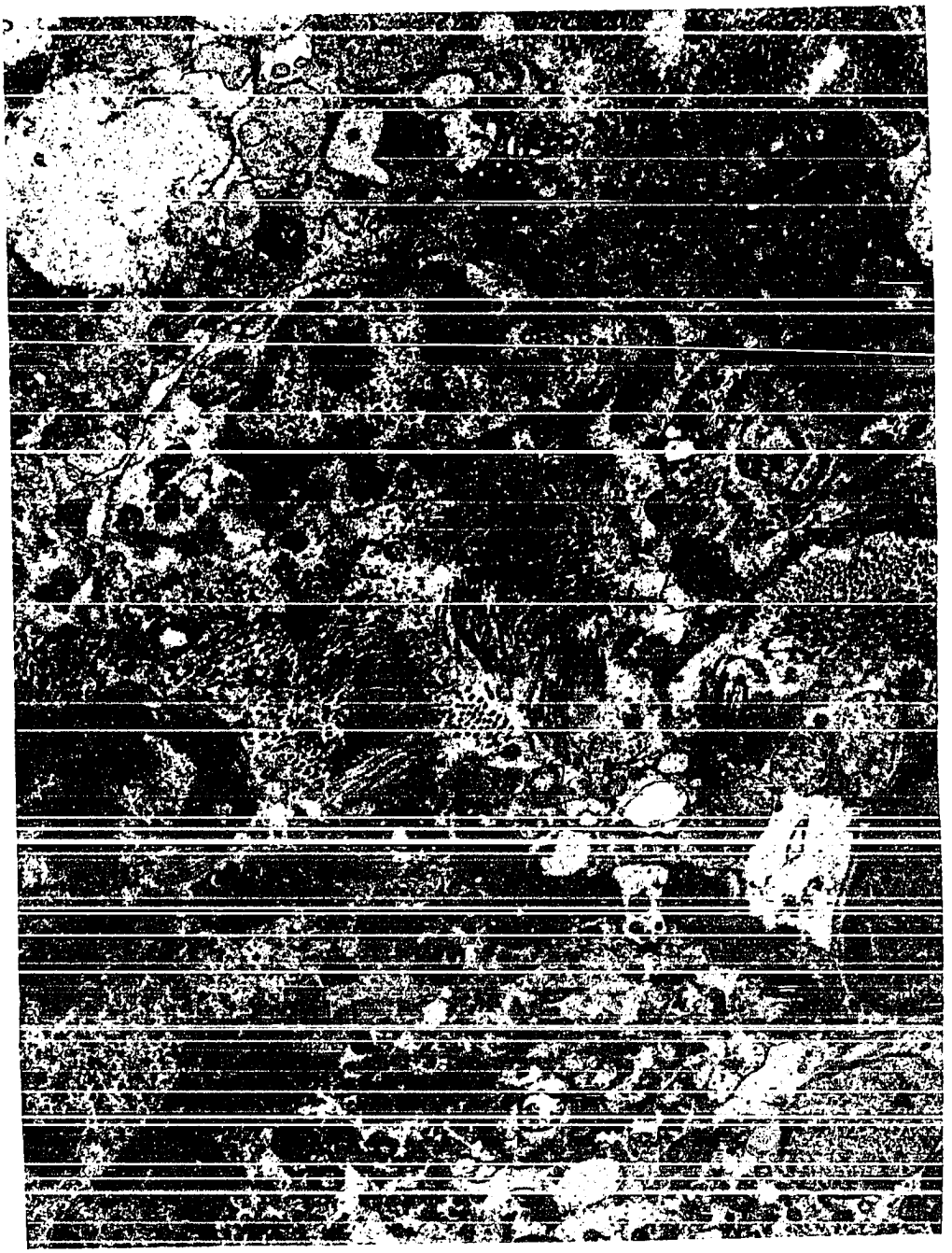


Figure 78. Hassel's corpuscle 4 DPI, Trial II. Cystic (C), squamous (S), and swollen epithelial cells are present. Clumped, marginated chromatin, nucleocapsids in the karyoplasm and cytoplasm, cytoplasmic edema, cytoplasmic vacuoles (some containing virions), and swollen mitochondria are present in these cells. x4,000

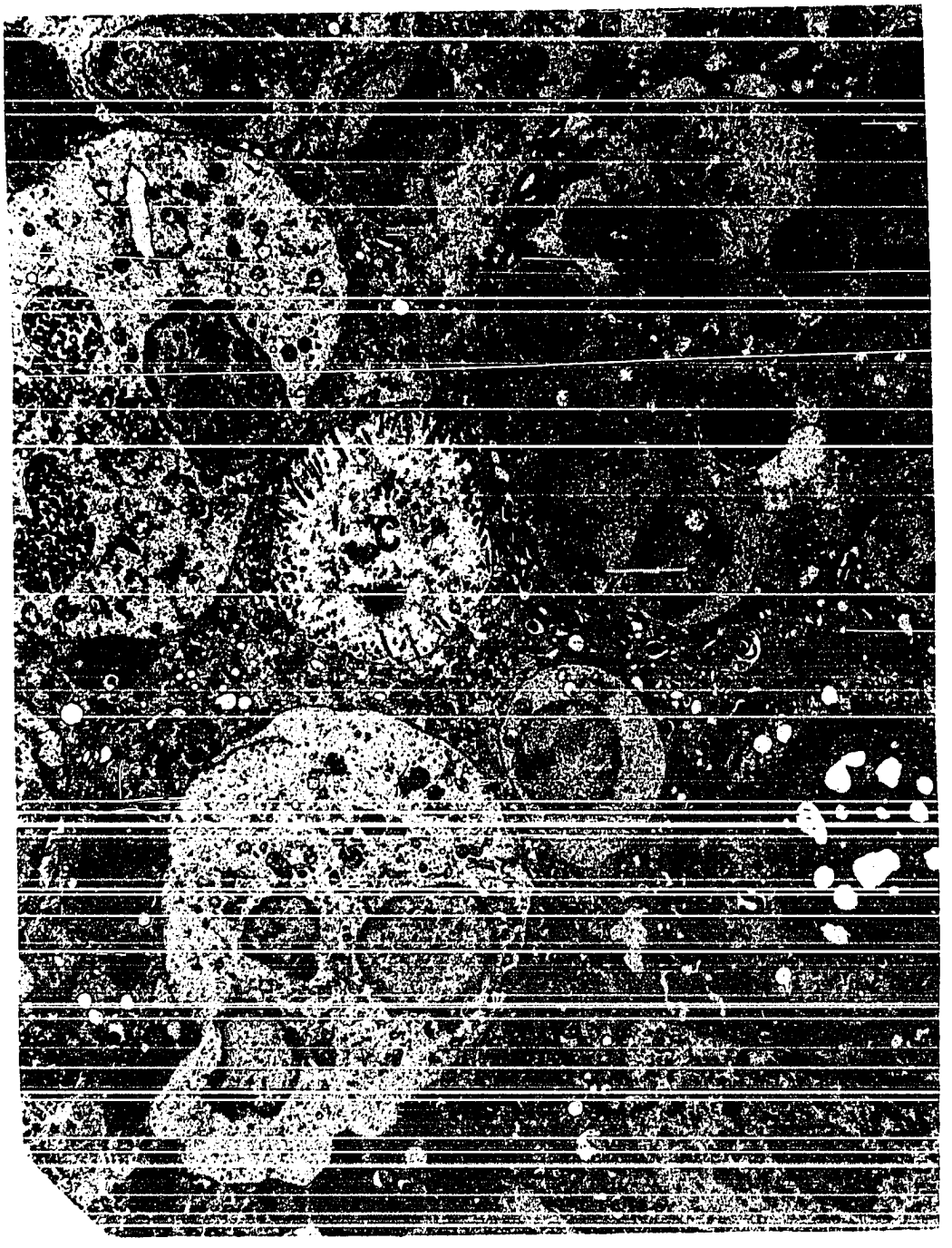
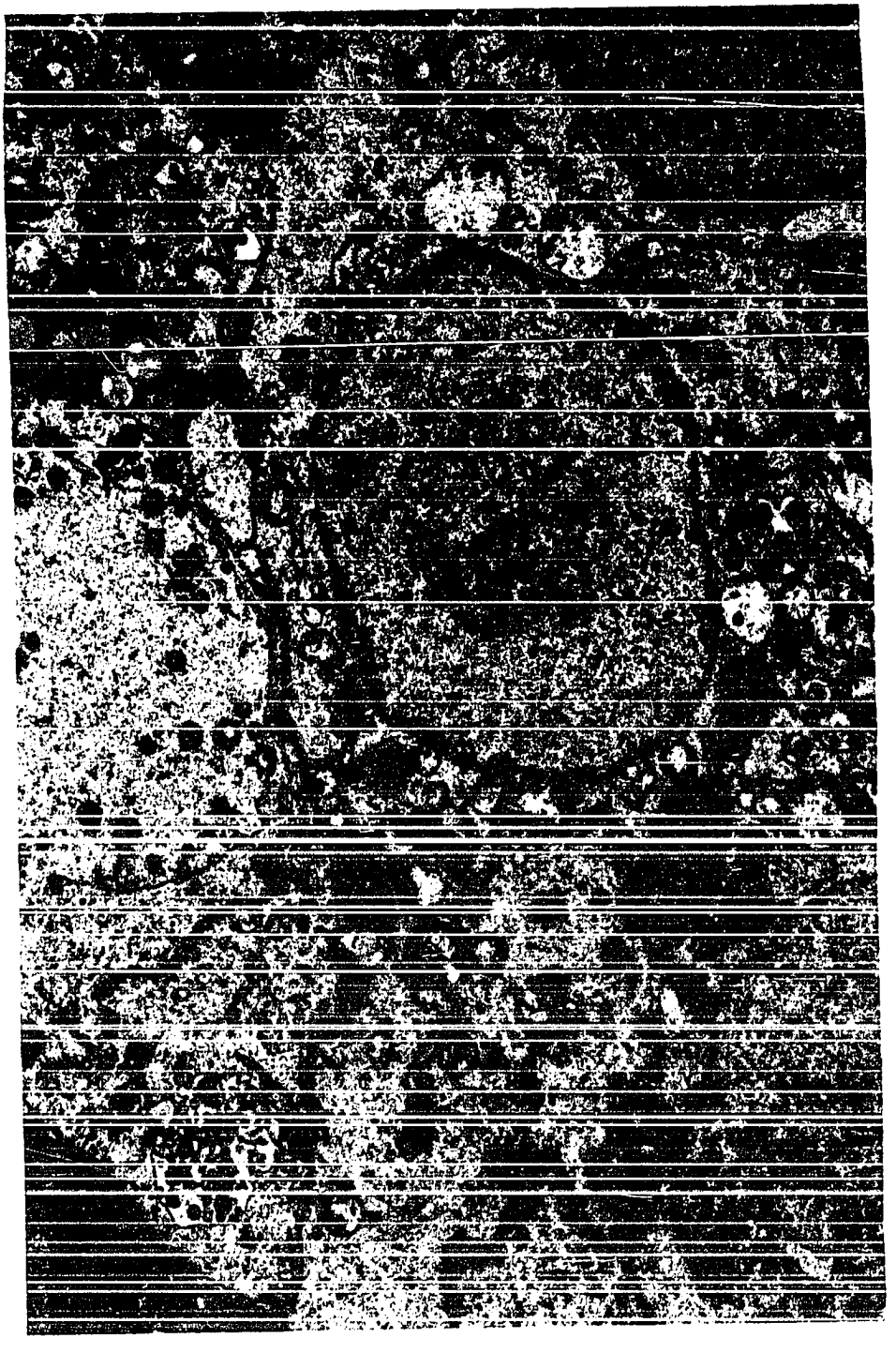


Figure 79. Hassel's corpuscle 4 DPI, Trial II. Mitochondria are swollen, and most of the cristae are missing. Chromatin is clumped and marginated, and nucleocapsids are present in the karyoplasm of the cystic epithelial cell. Virions are present in the cytoplasm of the adjacent epithelial cell. x10,800



Nucleocapsids were found in the nuclei and in the cytoplasm around dense bodies of these cells. Occasionally a virion was found in a cytoplasmic vacuole of a lymphocyte (Fig. 80). Macrophages were seen adhering to the vascular endothelium, and mast cells in the surrounding tissue were partially degranulated (Fig. 81). Five DPI diffuse necrosis and degeneration of lymphocytes and epithelial cells were observed in the thymic medulla, and the smaller cortical lymphocytes were also degenerate.

Trial III

Electron microscopy

Liver Lesions of the liver were initially detected 36 hours post-inoculation (HPI). At this time increased numbers of macrophages were observed in hepatic sinusoids sticking to the endothelium. Sinusoidal endothelial cells were swollen and contained numerous pinocytic vesicles or caveolae intracellulares (Fig. 82). By 48 HPI many macrophages had migrated into the space of Disse or were between endothelial junctions. Macrophages in the sinusoids contained large phagolysosomes, and nucleocapsids were in the nuclei of some macrophages. Irregular blebs of endothelium projected into the sinusoids. By 60 HPI many sinusoids were filled with degenerate macrophages (Fig. 83). Nucleocapsids were present among the clumped-marginated chromatin, and virions were present in

Figure 80. Thymic medulla 4 DPI, Trial II. Cytolysis and karyorrhexis of lymphocytes have occurred. Nucleocapsids are present around dense cytoplasmic bodies, and several virions are present in the cytoplasm of one lymphocyte. x9,500

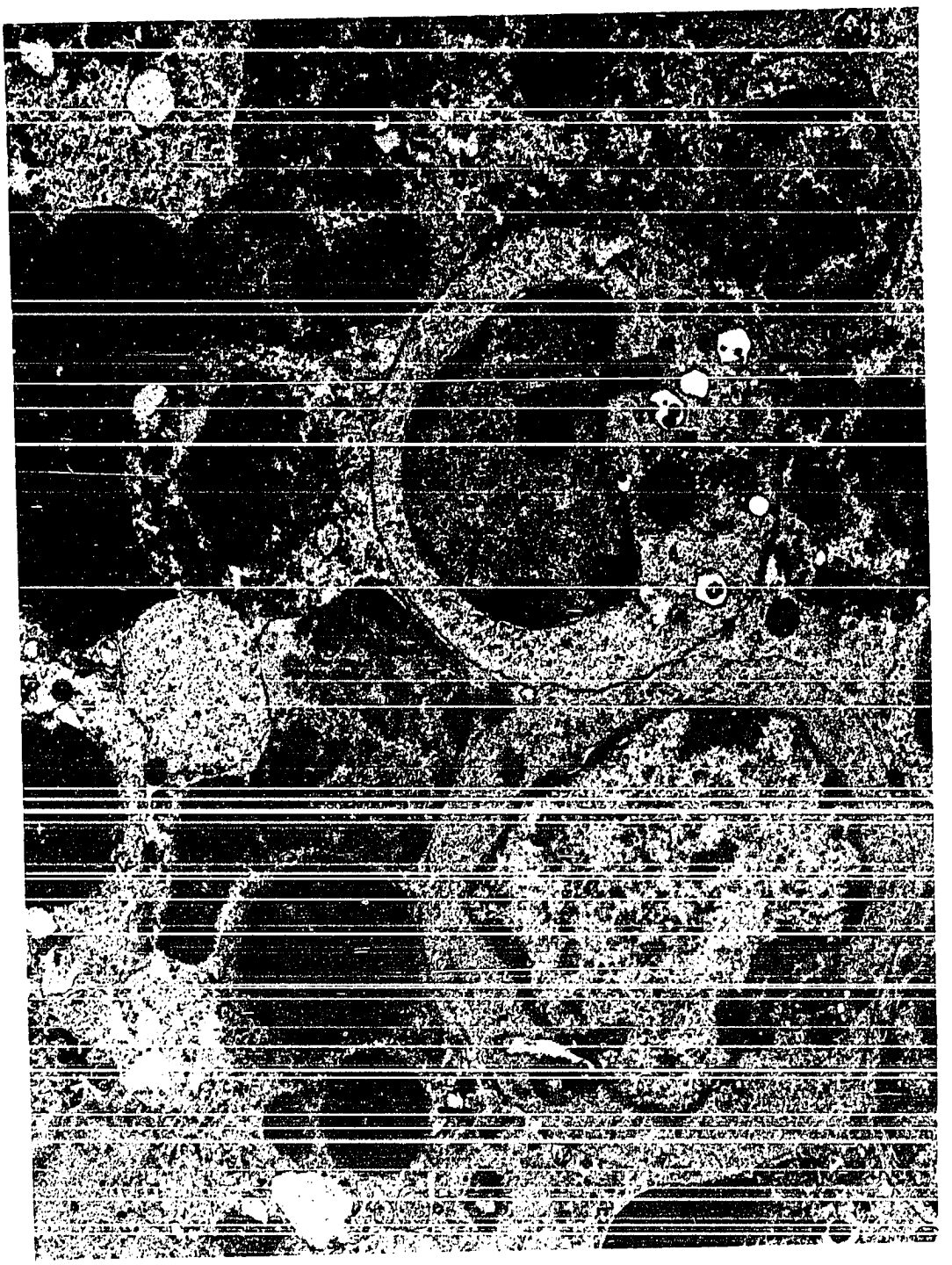


Figure 81. Thymic vessel 4 DPI, Trial II. Endothelium is swollen; and heterophils, mononuclear cells, and a basophil are present on the endothelium. The mast cell in the surrounding tissue is partially degranulated. x6,000

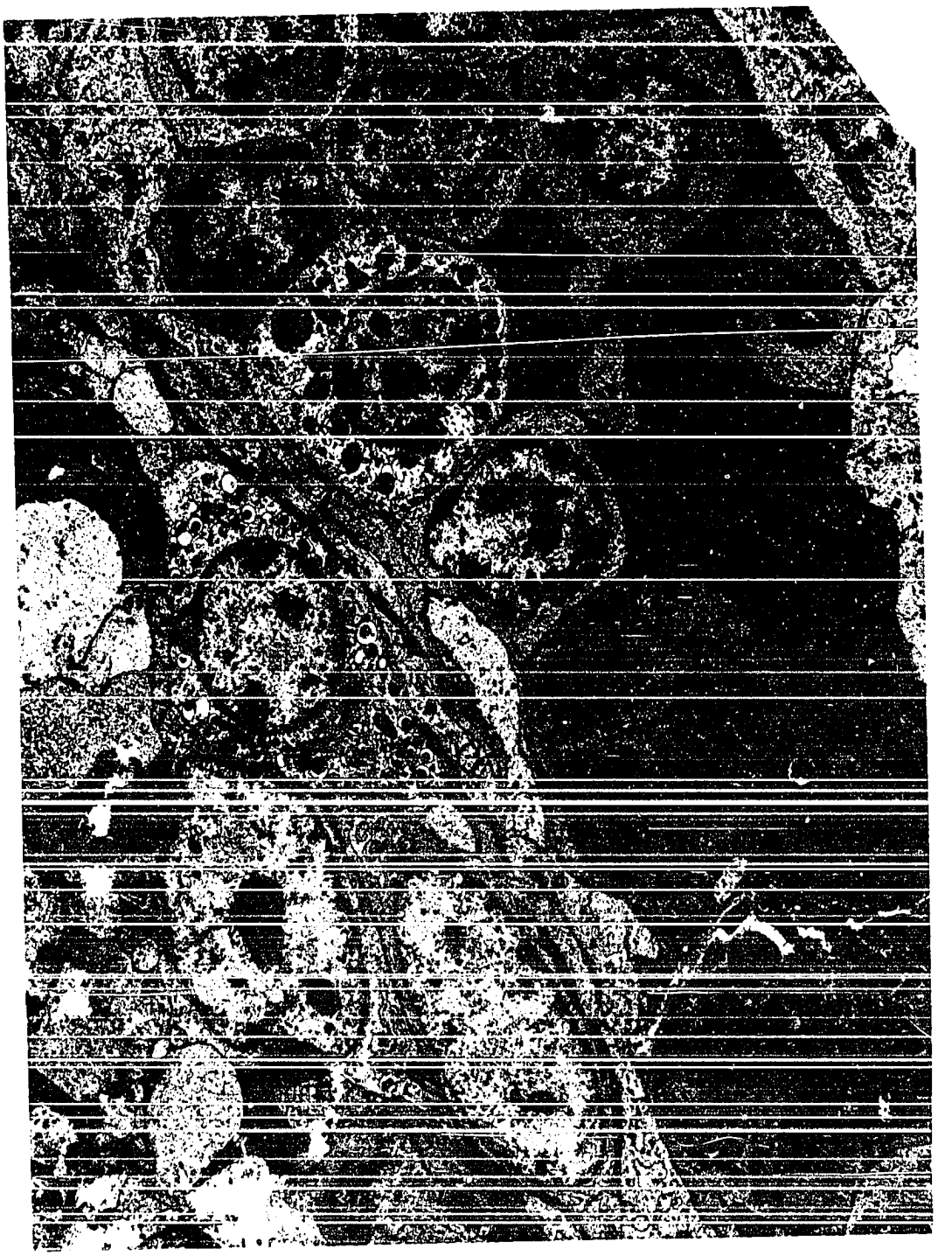


Figure 82. Hepatic sinusoid 36 HPI, Trial III. A macrophage is adhering to the sinusoidal endothelium. Phagocytized debris and a swollen mitochondria are present in the cytoplasm of the macrophage. Many pinocytic vacuoles are present in the endothelial cytoplasm, and blebs of endothelium project into the sinusoid. x11,000

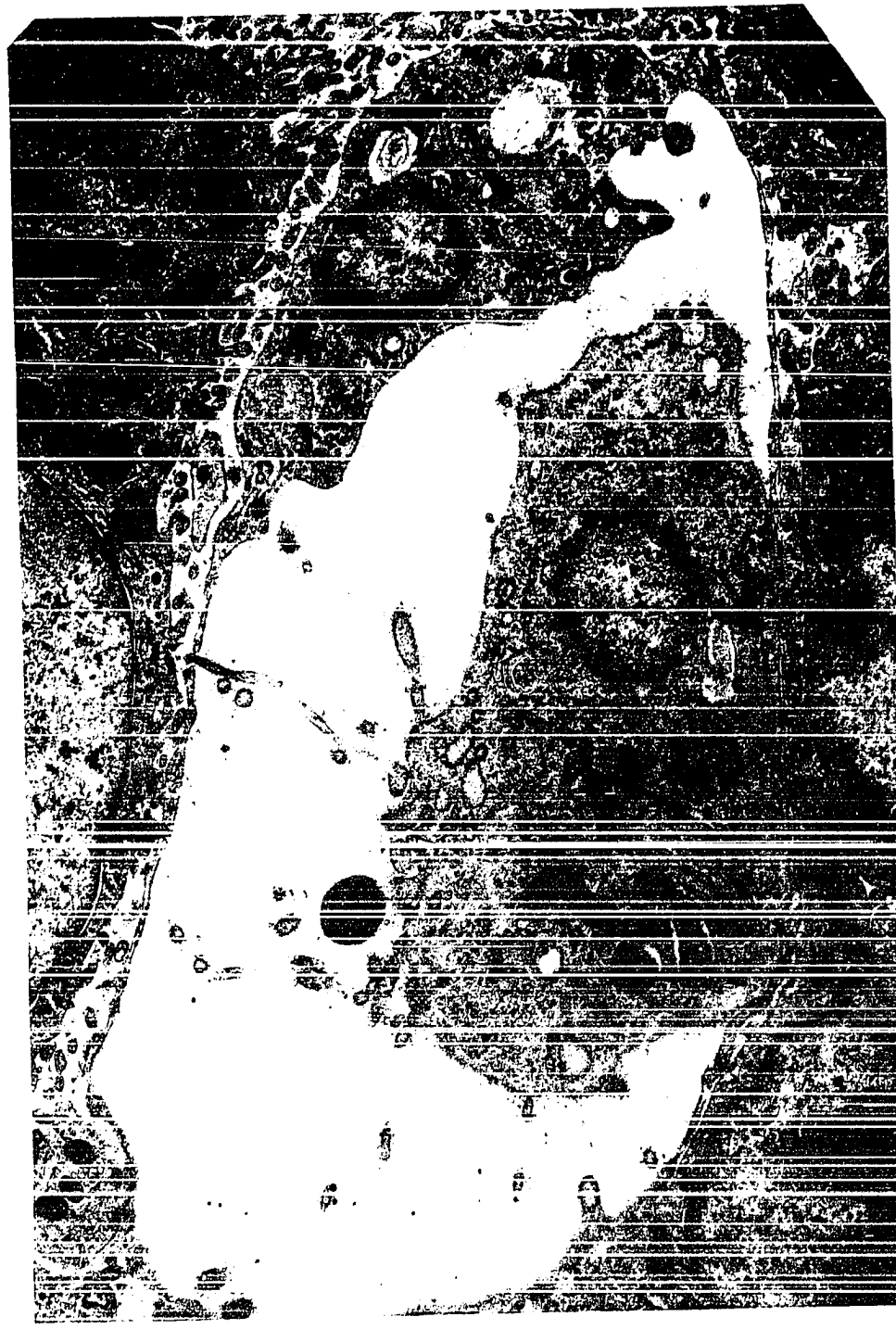


Figure 83. Hepatic sinusoid 60 HPI, Trial III, containing degenerate macrophages. Liposomes are present in the hepatocyte. x9,900

cytoplasmic vacuoles of these macrophages (Fig. 84). Hepatic microvilli, which extended into biliary canaliculi or into the space of Disse (Fig. 85), and microvilli on bile duct epithelium were blunted. Cytoplasm of hepatocytes was pale, and the endoplasmic reticulum was dilated. Virions were present in cytoplasmic vacuoles. Chromatin was clumped and marginated, and nucleocapsids were dispersed throughout the nuclei (Fig. 86).

Ultrastructural lesions observed 72 and 84 HPI were similar. Foci of necrotic hepatocytes and bile duct epithelium were seen. Nucleocapsids and virions were scattered among the nuclear and cytoplasmic detritus of these cells. In the cytoplasm of adjacent hepatocytes and bile duct epithelium, mitochondria were swollen, the cristae were broken, and the matrix was electron translucent. Large fat liposomes were present in the hepatocytes (Fig. 87). Smooth and rough endoplasmic reticulum was dilated, and many ribosomes were detached from the rough endoplasmic reticulum. Glycogen granules were difficult to find in affected hepatocytes. Virions with dense cores were in cytoplasmic vacuoles of hepatocytes (Fig. 88) and biliary epithelium (Fig. 89). Some virions were in the endoplasmic reticulum near the Golgi complex. A dark, electron dense material filled the space between the capsid and the envelope (Fig. 88), and occasionally two nucleocapsids were enclosed in one envelope. In the nuclei the chromatin

Figure 84. Hepatic macrophage 60 HPI, Trial III. Chromatin is clumped and marginated. Fine granular material, nucleocapsids, and primary bodies are in the nucleus. The perinuclear cisterna is irregular, and the nuclear contour is irregular. x34,400

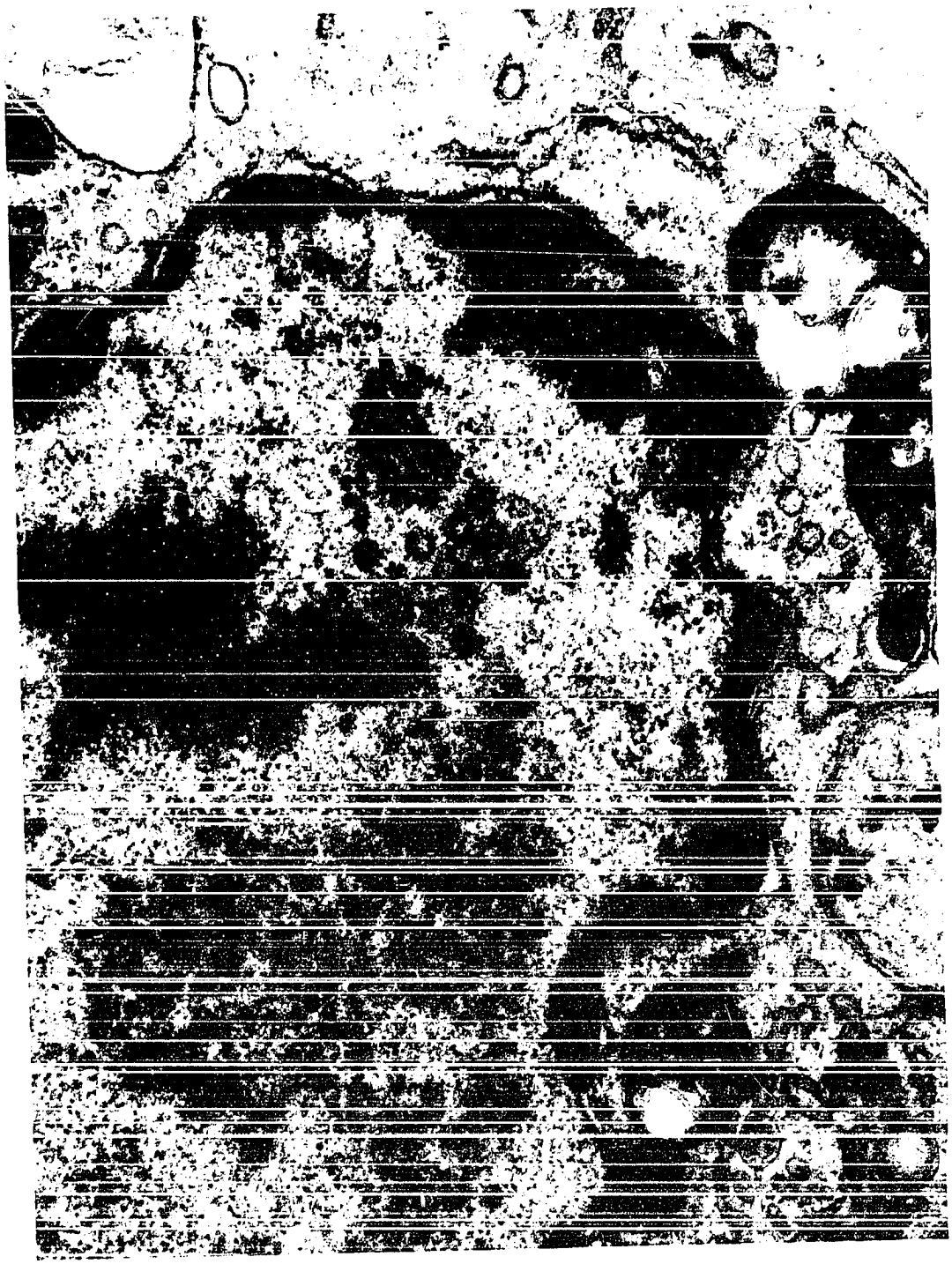


Figure 85. Hepatic sinusoid 60 HPI, Trial III. Two macrophages are in the space of Disse, and one is projecting through an endothelial junction into the sinusoid. Microvilli are missing on most of the hepatocytic surface bordering the space of Disse. x10,300

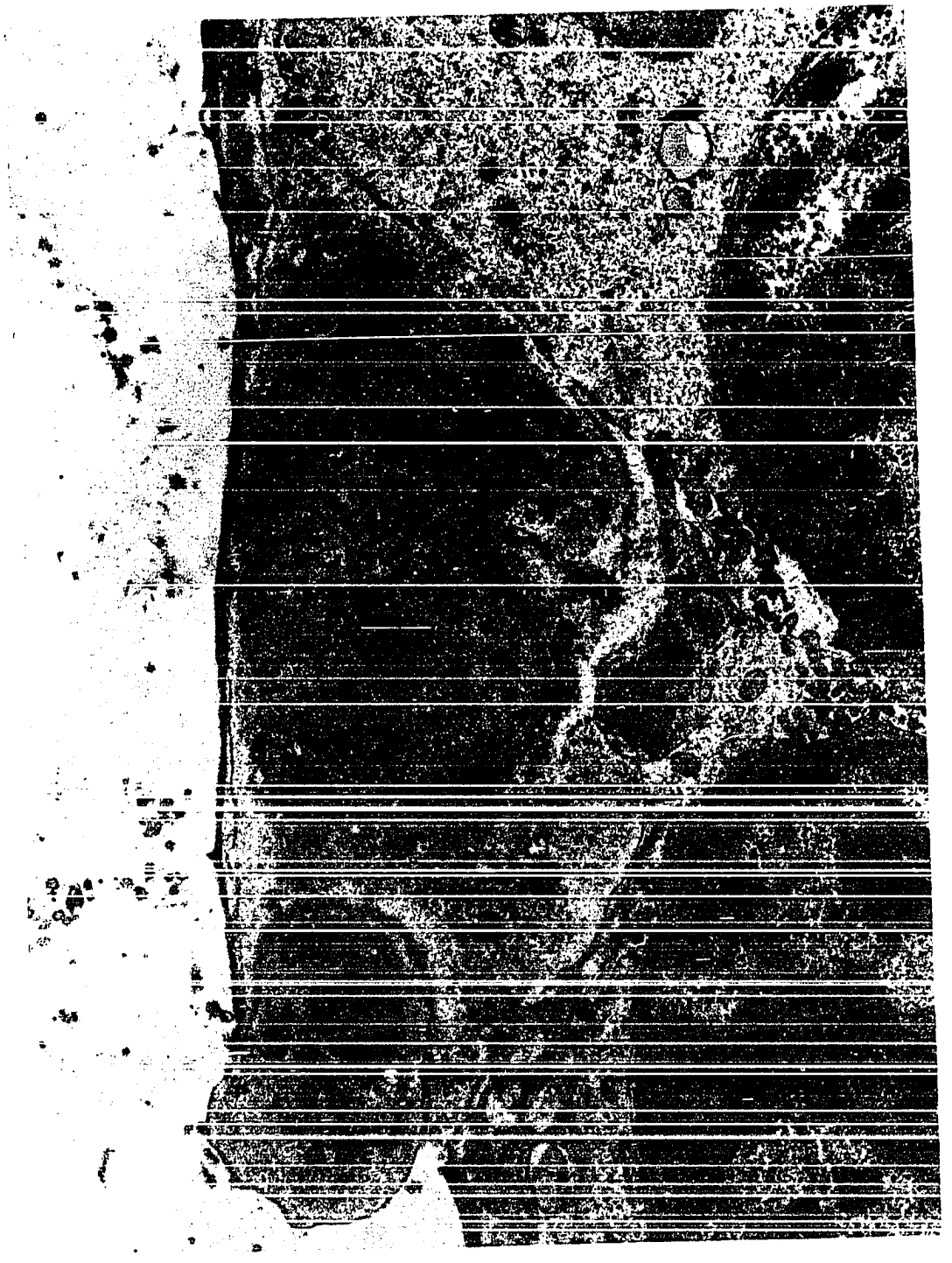


Figure 86. Liver 60 HPI, Trial III. Macrophages are present between hepatocytes. Chromatin is clumped and marginated in the hepatocytes, and contour of the nuclear membranes is irregular. x7,700

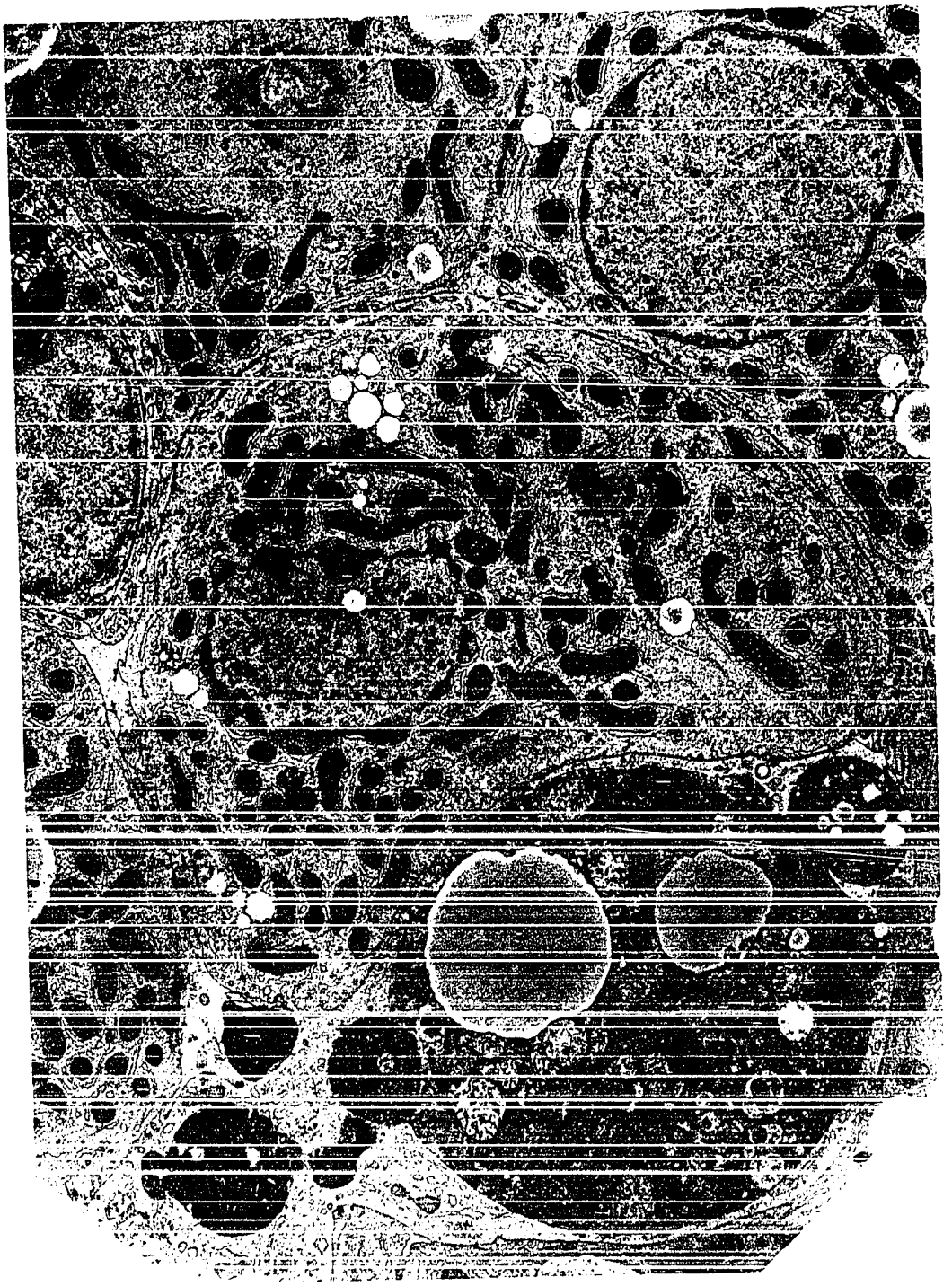


Figure 87. Hepatocyte 72 HPI, Trial III. Mitochondria are swollen, and a large liposome is in the cytoplasm. The nuclear chromatin is clumped and marginated, and fine granular material is present in the center of the nucleus. x17,000

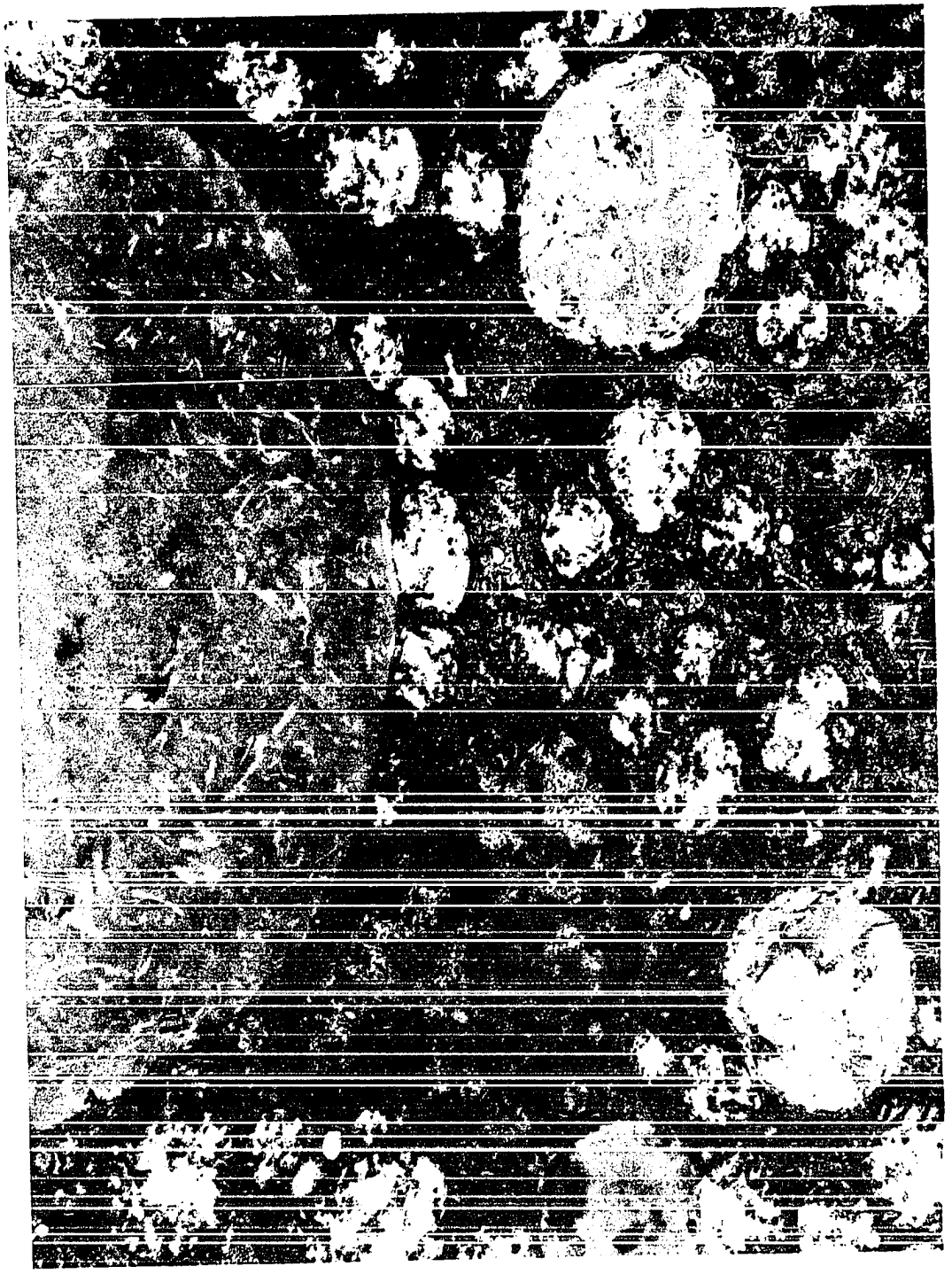
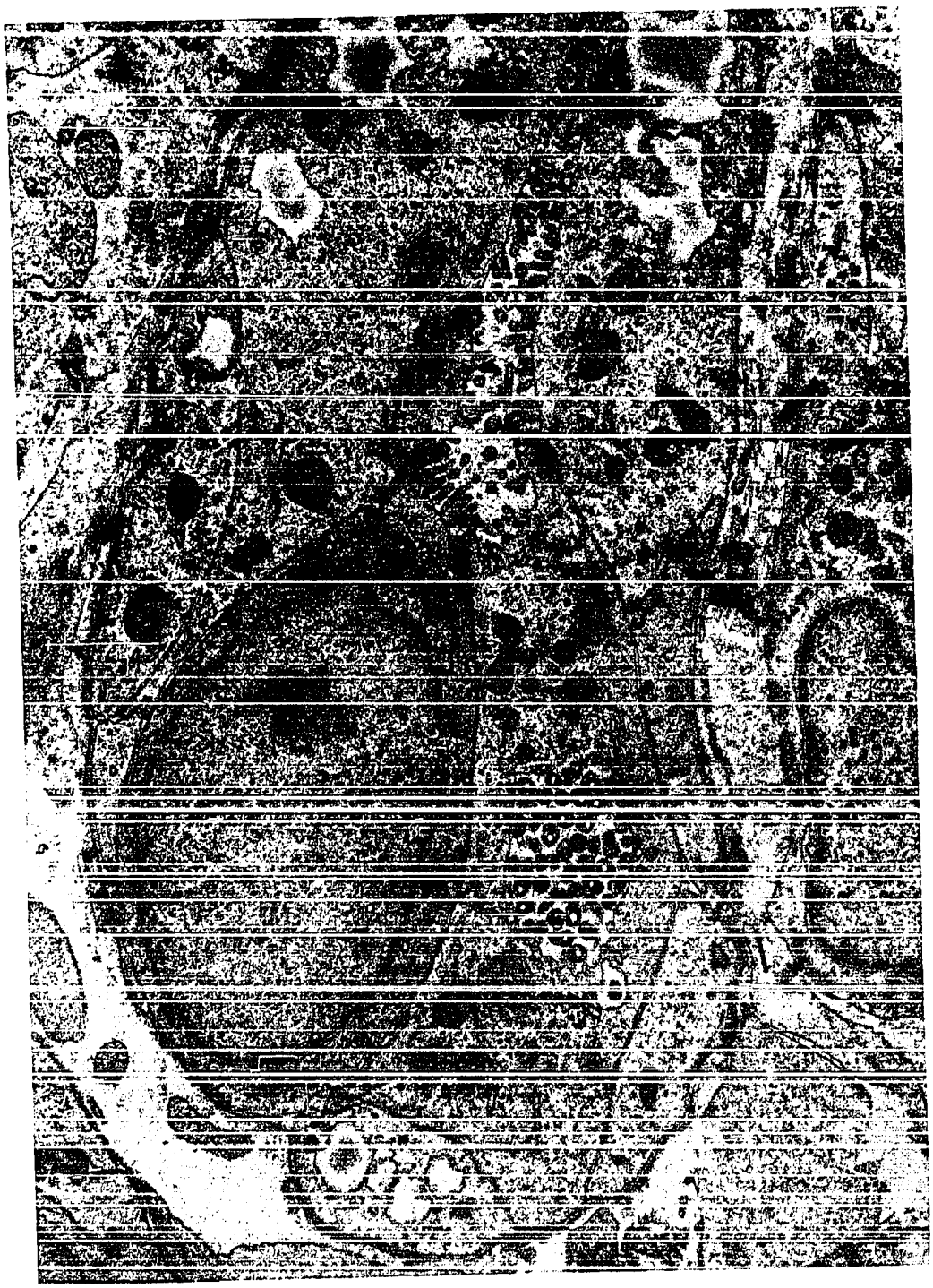


Figure 88. Hepatocyte 72 HPI, Trial III. Virions are present in the cytoplasmic vacuoles. Hepatic villi which extend into the biliary canaliculi are blunted. x17,500

Figure 89. Bile duct epithelium 84 HPI, Trial III. Microvilli extending into the duct are blunted, and virions are present in a cytoplasmic vacuole. x10,800



was clumped and marginated; and fine granular material, nucleocapsids, and clumped chromatin formed intranuclear inclusion bodies (Figs. 87 and 90). Nucleocapsids were often found in clusters and contained three different types of cores: (1) small dense spherical cores, (2) hollow spherical cores, and (3) bar-shaped cores. Clusters of small dense particles (primary bodies) (Fig. 84) and filaments were occasionally found in nuclei of affected cells (Fig. 90). Nuclear membrane contained irregular folds, and small segments of reduplicated inner nuclear membrane were found projecting into the perinuclear cisternae or into the karyoplasm (Fig. 91). Occasionally a nucleocapsid was found partially budded through the inner nuclear membrane.

Trial IV

Effect of lead on the susceptibility of ducks to duck plague virus

The mean packed cell volumes (PCV) of ducks that received different numbers of lead shot varied during the experiment. Ducks given 0, 8, and 16 lead shot had a decline in their PCV the first 3 weeks post-inoculation and a slight increase in PCV the fourth week. Ducks given four lead shot had a drop in their PCV the first week post-inoculation, a rise in PCV the second week post-inoculation, a decline the third week, and a slight increase the fourth week post-inoculation (Fig. 92).

Figure 90. Hepatocyte 84 HPI, Trial III. Chromatin is clumped and marginated. Clusters and scattered nucleocapsids are present in the karyoplasm. Three filaments are also in the karyoplasm. The material in the central karyoplasm makes up the inclusion body seen histologically. x31,200

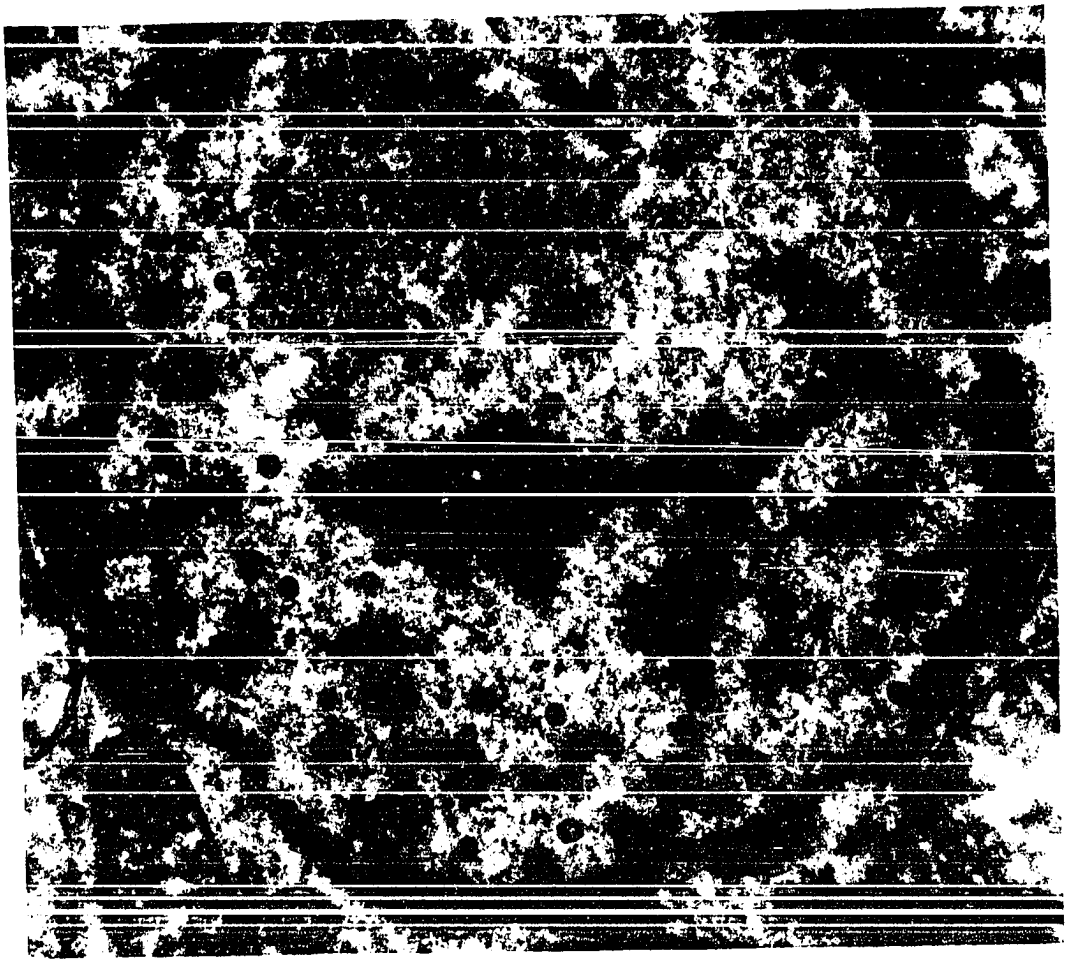
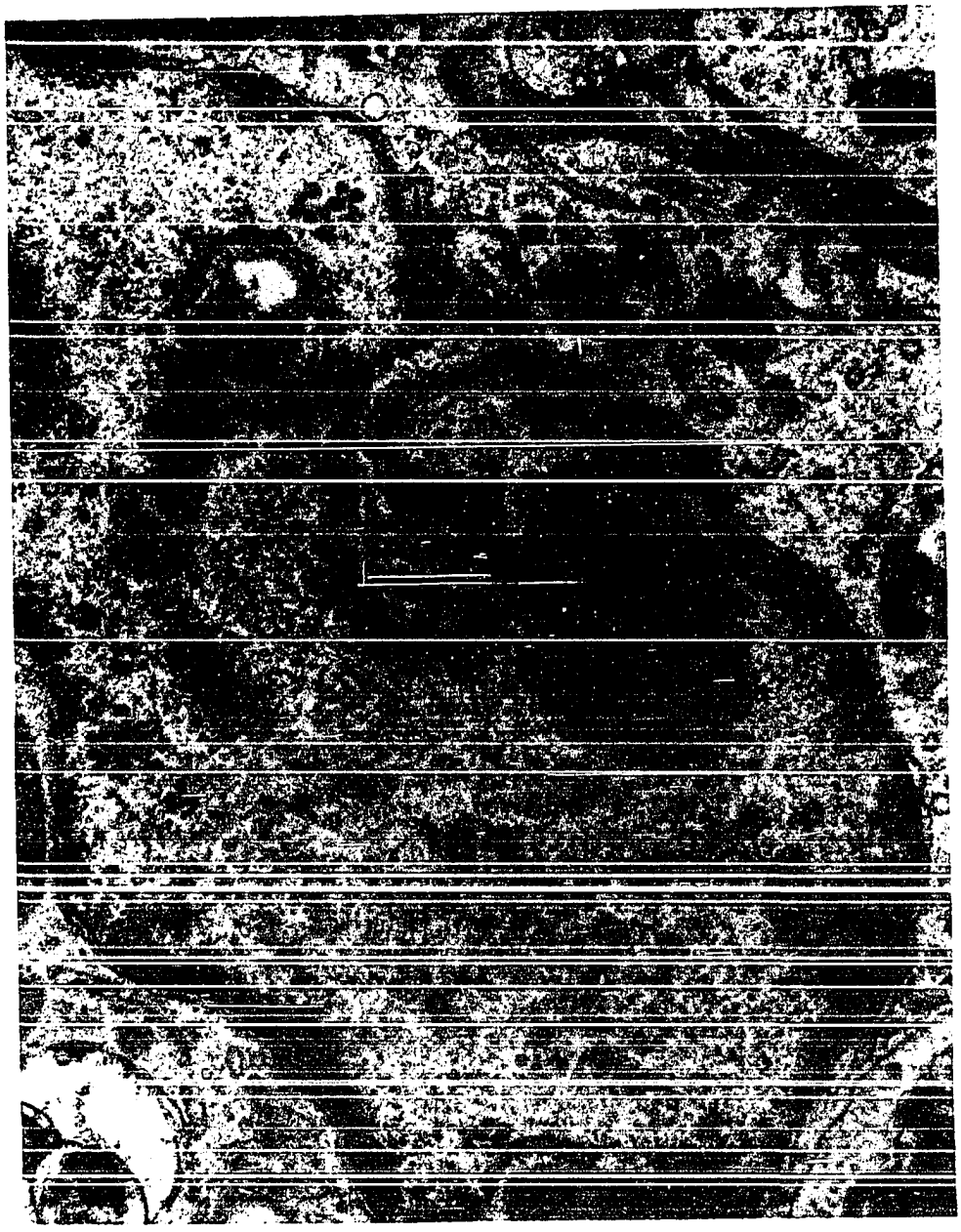


Figure 91. Hepatocyte 84 HPI, Trial III. Irregular folds are present in the nuclear membrane, and small segments of reduplicated inner nuclear membrane project into the perinuclear cisterna. An occasional nucleocapsid is present in the karyoplasm. x26,000



An analysis of variance did not reveal a significant difference in the PCV caused by the number of lead shot the ducks received.

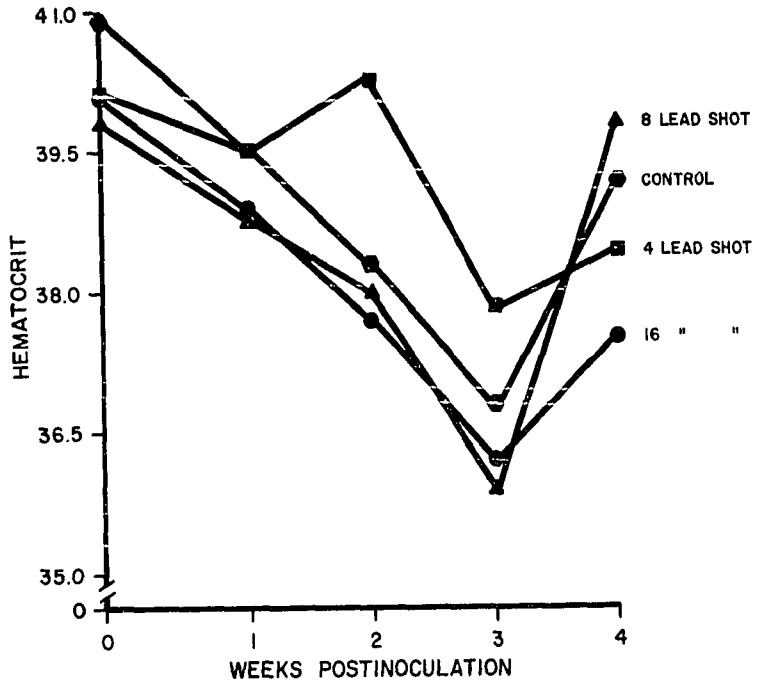
Ducks were initially weighed the first week post-inoculation. Using this weight as a base weight for each group, a higher percent of weight gain was found 4 weeks post-inoculation in control ducks that was significantly different from the ducks given 4, 8, or 16 lead shot orally (Fig. 93).

Blood lead levels of individual ducks 1, 2, 3, and 4 weeks post-inoculation are given in Table 2. Mean blood lead levels increased markedly the first week post-inoculation in the three groups of ducks given lead shot. During the second, third, and fourth weeks post-inoculation mean blood lead levels decreased in ducks given 4 and 16 lead shot. In ducks given 8 lead shot the mean blood lead level increased to a peak 3 weeks post-inoculation and then declined (Fig. 94). A separate analysis of variance was run for each week post-inoculation. Significant differences were found in blood lead levels caused by the number of lead shot given orally for the first 3 weeks post-inoculation. No significant difference was found in the blood lead levels the fourth week post-inoculation.

Levels of lead found in the liver, kidney, and bone of each duck at necropsy varied (Table 3). The treatment mean level of lead in the liver and bone increased as number of lead shot given orally was increased from 0 to 16. Mean renal lead levels also increased as the number of shot given orally

Figure 92. Trial IV. The average hematocrit in each group of ducks given 0, 4, 8, and 16 number 6 lead shot 1, 2, 3, and 4 weeks post-inoculation

Figure 93. Trial IV. The mean total percent weight gain for each group of ducks given 0, 4, 8, or 16 lead shot orally determined 2, 3, and 4 weeks post-inoculation using the 1 week post-inoculation weight as a base weight


41.0
39.5
38.0
36.5
35.0
0
HEMATOCRIT
8 LEAD SHOT
CONTROL
4 LEAD SHOT
16 " "
0
1
2
3
4
WEEKS POSTINOCULATION

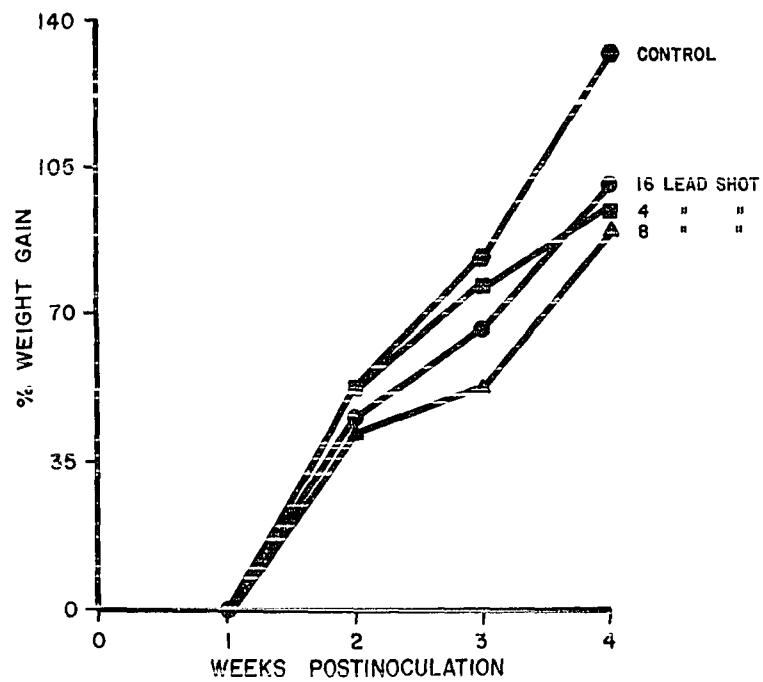

140
105
70
35
0
% WEIGHT GAIN
CONTROL
16 LEAD SHOT
4 " "
8 " "
0
1
2
3
4
WEEKS POSTINOCULATION

Table 2. Weekly blood lead levels in ppm after ducks were given lead shot orally

Duck number	Number of shot given	Weeks post-inoculation				
		0	1	2	3	4
1	16	0	2.0	1.4	1.0	0.15
2	16	0	2.0	1.4	1.0	0.1
3	16	0	3.4	1.3	0.6	0.1
4	16	0	3.4	1.3	0.6	0.2
5	16	0	2.95	0.55	0.6	0.1
6	16	0	2.95	0.55	0.6	0.6
7	16	0	4.25	1.95	1.8	1.4
8	16	0	4.25	1.95	1.8	1.3
9	16	0	2.65	0.55	0.95	1.6
10	16	0.1	2.65	0.55	0.95	0.35
11	8	0.1	0.6	1.0	1.5	0.5
12	8	0.1	0.6	1.0	1.5	0.5
13	8	0.5	0.6	2.0	0.9	0.3
14	8	0.5	0.6	2.0	0.9	0.2
15	8	0.5	1.0	0.9	1.2	0.35
16	8	0.1	1.0	0.9	1.2	1.25
17	8	0.1	0.7	0.6	0.9	0.9
18	8	0.1	0.7	0.6	0.9	0.4
19	8	0	1.6	0.9	0.8	0.3
20	8	0	1.6	1.0	0.8	0.3
21	4	0	0.95	0.85	0.7	0.2
22	4	0.15	0.95	0.85	0.7	0.5
23	4	0.15	0.6	0.65	0.35	0.35
24	4	0.15	0.6	0.65	0.35	0.45
25	4	0	1.5	0.35	0.55	0.5
26	4	0	1.5	0.35	0.55	0.45
27	4	0	1.5	0.5	0.35	0.7
28	4	<0.1	0.9	0.5	0.35	0.6
29	4	<0.1	0.55	0.5	0.35	0.6
30	4	<0.1	0.55	0.5	0.35	0.2
31	0	0.2	0.15	0.15	0.2	0.15
32	0	0.2	0.15	0.15	0.2	0.2
33	0	0	0.15	0.1	0.15	0.15
34	0	<0.1	0.15	0.1	0.15	0.2
35	0	<0.1	0.25	0.25	0.2	0.2
36	0	<0.1	0.25	0.25	0.2	0.15
37	0	0.15	0.25	0.25	0.15	0.15
38	0	0.15	0.25	0.25	0.15	0.15
39	0	0.15	0.15	0.25	0.3	0.2
40	0	-[a]	0.15	0.25	0.3	0.2

[a]Sample lost.

Table 3. Tissue lead levels in ppm found in ducks at necropsy

Duck number	Number lead shot given	Liver	Kidney	Bone
1	16	4.2	4.0	210.5
2	16	-[a]	-	-
3	16	9.5	12.5	285.7
4	16	-	6.5	315.8
5	16	3.5	6.5	258.8
6	16	2.5	7.5	252.9
7	16	9.0	2.6	411.1
8	16	4.0	10.0	363.6
9	16	5.0	10.5	200.0
10	16	5.5	11.0	272.7
11	8	3.0	6.0	197.2
12	8	3.5	7.5	368.1
13	8	16.5	35.5	239.1
14	8	3.0	8.0	364.9
15	8	8.5	15.5	393.4
16	8	5.5	12.0	171.1
17	8	2.5	6.0	100.0
18	8	0.5	1.5	92.8
19	8	1.0	4.0	90.5
20	8	1.0	3.0	95.2
21	4	0.5	1.0	96.0
22	4	1.5	5.0	107.8
23	4	1.0	2.0	55.0
24	4	0.5	2.5	33.0
25	4	2.0	4.5	76.5
26	4	-	1.0	46.2
27	4	1.5	6.0	46.1
28	4	1.0	1.0	99.6
29	4	1.0	2.0	66.4
30	4	1.0	1.5	55.0
31	0	0	0	16.2
32	0	1.0	0	21.3
33	0	0	0	13.4
34	0	0	0	13.2
35	0	0	0	16.2
36	0	0	0	44.9
37	0	0	0	18.3
38	0	0	0	13.8
39	0	0	0	14.8
40	0	0	0	31.3

[a]Sample lost.

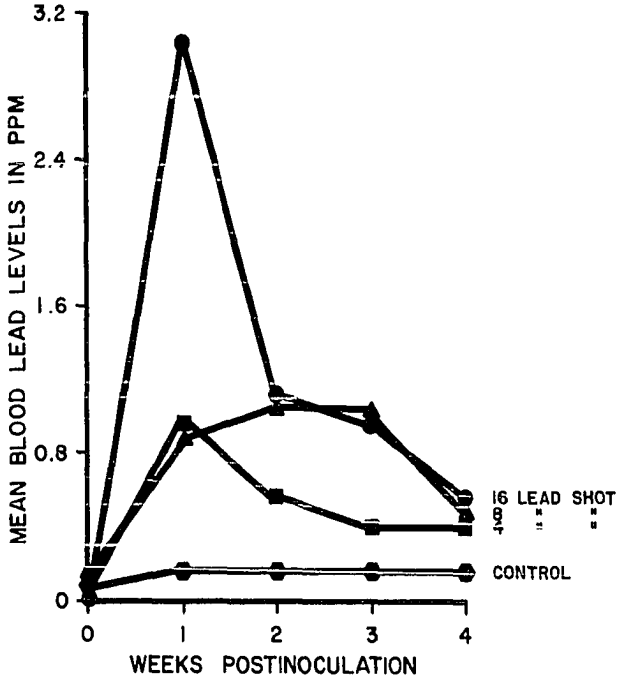
was increased from 0 to 4 to 8 lead shot, but the mean level found in the kidney of the ducks receiving 16 lead shot was less than that found in the ducks receiving 8 lead shot orally (Table 4). An analysis of variance demonstrated a significant difference in the levels of lead in the liver, kidney, and bone due to the number of lead shot given orally. A least significant difference test for liver and kidney revealed this difference was between the groups of ducks given 0 or 4 lead shot and those given 8 or 16 lead shot. Using the same test for bone, the mean for every group was significantly different from every other group except for the control group and the group given only four lead shot.

Death occurred sooner in ducks given lead shot orally than in control ducks receiving the same amount of DPV. All of the ducks given 4, 8, or 16 number 6 lead shot 4 weeks prior to oral inoculation of 0.5 ml of DPV (10^4 $DELD_{50}$ per ml) died by the fourth DPI. One of the control ducks died on the third DPI, five on the fourth DPI, two on the fifth DPI, and two on the seventh DPI (Fig. 95). An analysis of variance demonstrated a significant difference in the mean day of death between the control group and each group given lead shot.

Gross lesions observed in the ducks receiving lead orally and the control ducks were similar to those described in the section on macroscopic lesions. The number of lead shot found

Figure 94. Trial IV. The treatment mean lead levels in ppm per ml of blood for ducks receiving 0, 4, 8, or 16 number 6 lead shot orally determined 1, 2, 3, and 4 weeks post-inoculation

Figure 95. Trial IV. Four groups of 10 ducks each were given 0, 4, 8, or 16 lead shot 4 weeks prior to oral inoculation of 0.5 ml of 10^4 $DELD_{50}$ DPV. The total percent mortality for each group of ducks was plotted


3.2
2.4
1.6
0.8
0
MEAN BLOOD LEAD LEVELS IN PPM
16 LEAD SHOT
8 " "
4 " "
CONTROL
0
1
2
3
4
WEEKS POSTINOCULATION

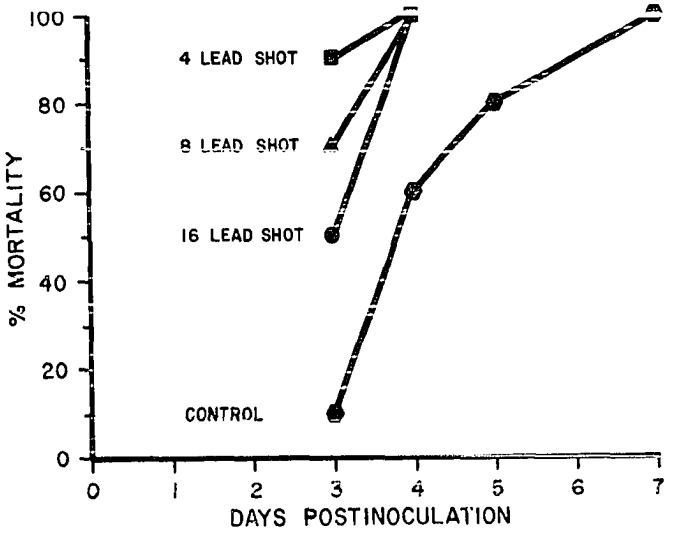

100
80
60
40
20
0
% MORTALITY
4 LEAD SHOT
8 LEAD SHOT
16 LEAD SHOT
CONTROL
0
1
2
3
4
5
6
7
DAYS POSTINOCULATION

Table 4. Mean lead levels in ppm found in tissues of ducks at necropsy

Tissue	Number of shot received			
	0	4	8	16
Bone	20.3	68.2	211.2	285.7
Liver	0.1	1.1	4.5	5.4
Kidney	0	2.7	9.9	7.9

in the ventriculus of each duck is given in Table 5. Microscopic lesions observed were also similar to those described in the section on microscopic lesions. Some of the intranuclear inclusion bodies in hepatocytes of ducks receiving lead orally contained small flakes of acid-fast material.

Trial V

Effect of lead on the susceptibility of ducks to duck plague virus

Levels of lead found in the liver, kidney, and bone of each duck collected at necropsy are given in Table 6. The treatment mean lead levels found in each tissue are given in Table 7. Mean lead levels found in bone, liver, and kidney increased as the number of lead shot given orally increased from 0 to 4 to 8 to 16. An analysis of variance for each tissue demonstrated significant differences in the levels of lead in

Table 5. Number of lead shot found in the ventriculus of ducks at necropsy

Duck number	Number of shot received	Number of shot recovered
1	16	16
2	16	16
3	16	14
4	16	14
5	16	16
6	16	15
7	16	16
8	16	11
9	16	16
10	16	12
11	8	8
12	8	5
13	8	8
14	8	7
15	8	8
16	8	6
17	8	8
18	8	8
19	8	4
20	8	5
21	4	1
22	4	3
23	4	3
24	4	1
25	4	4
26	4	3
27	4	4
28	4	1
29	4	3
30	4	4
31	0	0
32	0	0
33	0	0
34	0	0
35	0	0
36	0	0
37	0	0
38	0	0
39	0	0
40	0	0

Table 6. Tissue lead levels in ppm found in ducks at necropsy

Duck number	Number lead shot given	Liver	Kidney	Bone
60	16	3.0	7.5	263.9
70	16	5.5	11.0	408.4
74	16	2.5	7.0	169.8
77	16	6.0	12.0	417.2
80	16	9.5	11.5	248.9
85	16	10.0	9.5	223.9
93	16	3.5	5.0	289.5
99	16	3.0	7.0	299.8
61	8	2.0	11.5	123.2
66	8	4.5	6.5	393.7
81	8	6.5	3.0	158.0
82	8	2.5	6.0	130.3
88	8	5.5	6.5	173.9
91	8	2.5	13.3	-[a]
95	8	2.0	2.5	125.2
97	8	5.0	3.5	129.3
64	4	2.0	6.0	82.6
65	4	0.5	7.5	99.4
71	4	1.0	-	-
73	4	3.5	3.0	115.8
78	4	3.0	5.5	58.1
86	4	5.0	7.0	155.1
89	4	0.5	6.0	25.2
96	4	0.5	-	18.6
63	0	0.5	0	-
68	0	0	0	0
69	0	0	0.5	0
76	0	0	0	0
79	0	0.5	0	0
84	0	0	0	1.2
92	0	0	0.5	0
98	0	0.5	0	0
62	0	0	0	9.3
67	0	0	0	0
72	0	0.5	0.5	0
75	0	0	0	0
83	0	0	0	0
87	0	0	0	0
90	0	0	0.5	0
94	0	0	0	0

[a]Sample lost.

Table 7. Mean lead levels in ppm found in tissues of ducks at necropsy

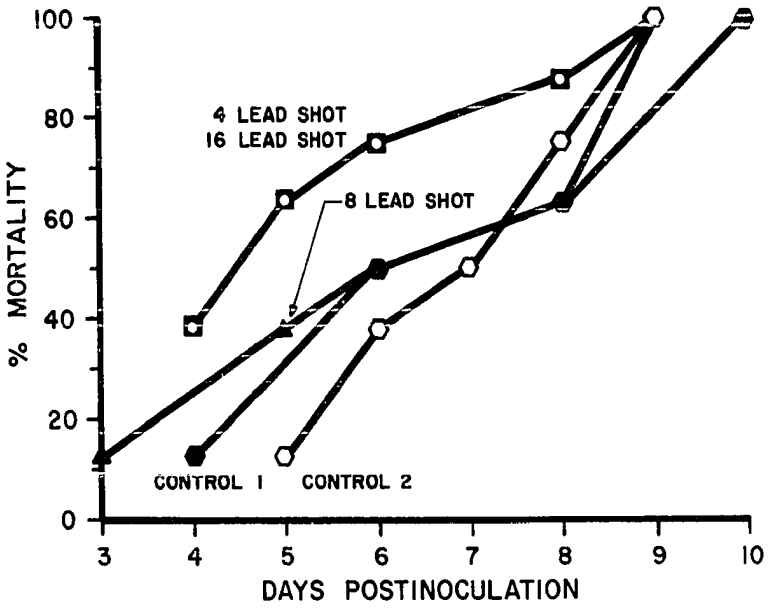
Tissue	Number of #6 lead shot given orally			
	0	4	8	16
Bone	1.3	79.3	176.2	290.2
Liver	0.06	2.0	3.8	5.4
Kidney	0.13	5.8	6.6	8.8

livers, kidneys, and bones. A least significant difference test revealed this difference in hepatic lead levels was between ducks given 0 or 4 lead shot and those given 8 or 16 lead shot. For the kidney the same results were found plus there was also a difference between the control ducks and those given 4 lead shot. Mean bone lead levels for each group were significantly different from every other group.

The total percent of ducks dead in each treatment group after 10^2 $DELD_{50}$ of DPV was given orally is presented in Fig. 96. An analysis of variance did not demonstrate a significant difference in the mean day of death between the control groups and groups given lead shot 4 weeks prior to oral inoculation with DPV.

The gross and microscopic lesions observed in these ducks were similar to those described in the previous section, and

Figure 96. Trial V. Five groups of eight ducks each were used. Control group #1 was left in an isolation room with the three groups of ducks given 4, 8, or 16 lead shot orally. Control group #2 was placed in another isolation room. Four weeks later all ducks were inoculated orally with 10^2 $DELD_{50}$ DPV orally, and the daily total percent mortality for each treatment group was plotted


100
80
60
40
20
0
% MORTALITY
4 LEAD SHOT
16 LEAD SHOT
8 LEAD SHOT
CONTROL 1
CONTROL 2
3
4
5
6
7
8
9
10
DAYS POSTINOCULATION

the number of lead shot found in the ventriculus is given in Table 8.

Trial VI

Gnotobiotic ducks

The spleen in the uninoculated gnotobiotic control duck was two-thirds as large as the spleen of the conventional control duck. Fewer lymphocytes were found around arterioles, and germinal centers were reduced in number in the spleen of the gnotobiotic duck. The decreased number of splenic lymphocytes caused the periarteriolar reticular sheaths of the gnotobiotic duck to be histologically prominent (Fig. 97) when compared with the conventional duck (Fig. 98).

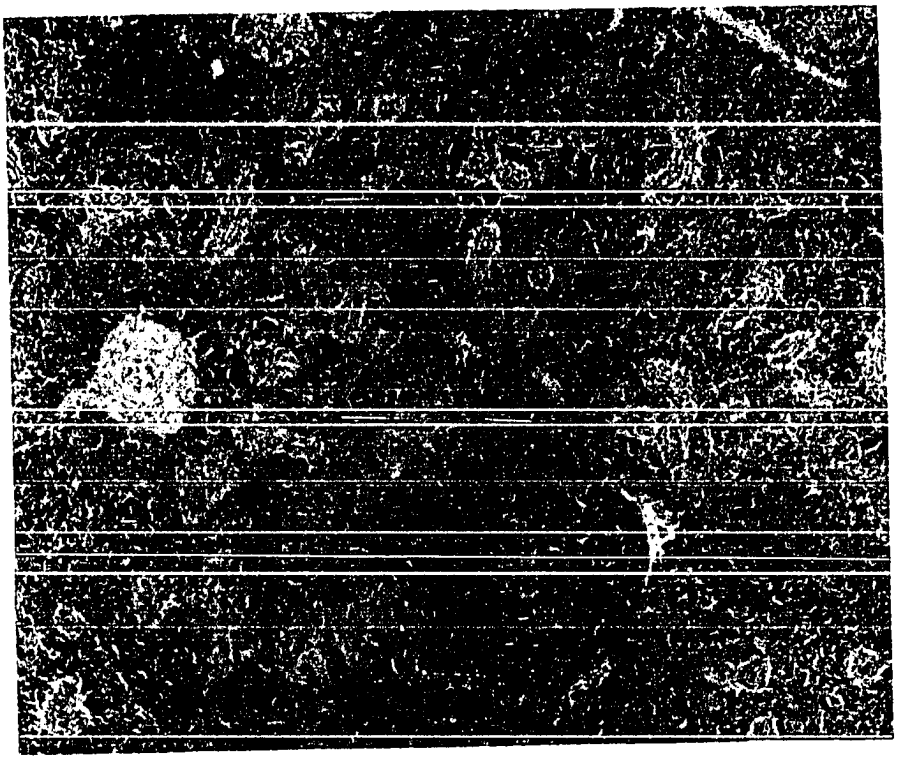
Intestinal annular bands could not be identified grossly in the conventional or gnotobiotic ducks. Lymphoid nodules were found in the intestinal submucosa of the conventional, but only an occasional nodule was found in the submucosa of the gnotobiot. Since annular bands could not be identified to examine histologically, Meckel's diverticulum and the esophageal-proventricular junction were examined to evaluate intestinal lymphoid development. Lymphoid nodules were found in both locations in the conventional duck (Figs. 99 and 101). Only irregularly shaped accumulations of lymphocytes were found at the esophageal-proventricular junction (Fig. 100) and in Meckel's diverticulum (Fig. 102) of the gnotobiotic duck.

Table 8. Number of lead shot found in the ventriculus of ducks at necropsy

Duck number	Number of shot received	Number of shot recovered
60	16	14
70	16	16
74	16	10
77	16	15
80	16	14
85	16	8
93	16	10
99	16	14
61	8	7
66	8	8
81	8	8
82	8	3
88	8	8
91	8	3
95	8	6
97	8	8
64	4	4
65	4	0
71	4	3
73	4	3
78	4	5
86	4	4
89	4	0
96	4	5
63	0	0
68	0	0
69	0	0
76	0	0
79	0	0
84	0	0
92	0	0
98	0	0
62	0	0
67	0	0
72	0	0
75	0	0
83	0	0
87	0	0
90	0	0
94	0	0

Figure 97. Spleen from a gnotobiotic control duck, Trial VI. Periarteriolar reticular sheaths are prominent, and few lymphocytes are present. H & E. x130

Figure 98. Spleen from a conventional control duck, Trial VI. Splenic lymphocytes are present, and the periarteriolar reticular sheaths are less prominent. H & E. x130



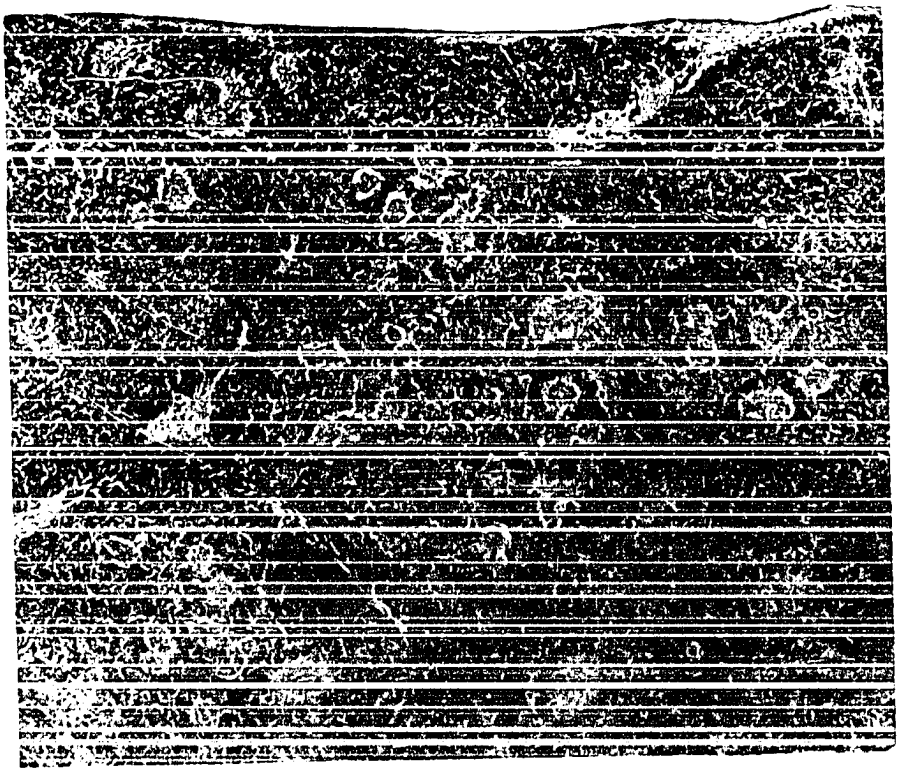
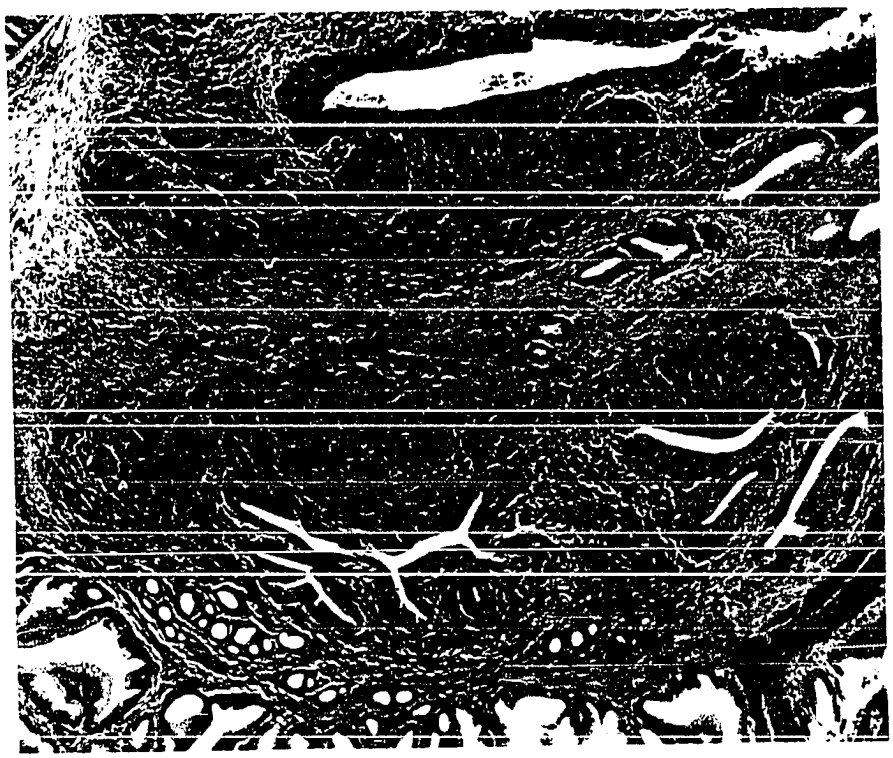


Figure 99. Esophageal-proventricular junction from a conventional control duck, Trial VI. Lymphoid nodules are present in the submucosa. H & E. x100

Figure 100. Esophageal-proventricular junction from a gnotobiotic control duck, Trial VI. Irregular accumulations of lymphocytes are present in the submucosa, but well-developed lymphoid nodules are absent. H & E. x100



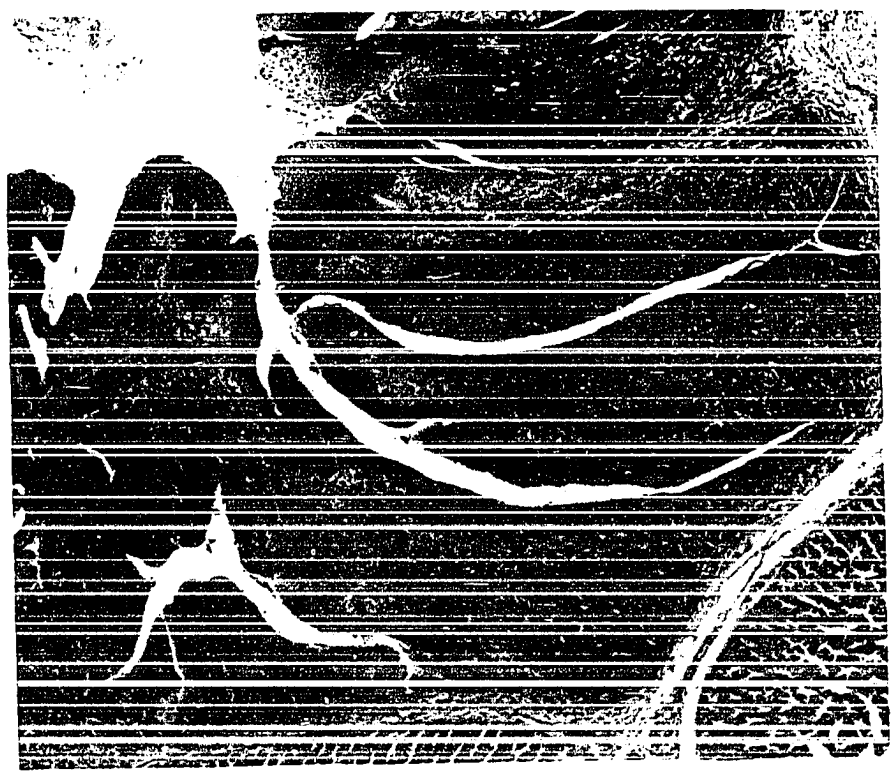
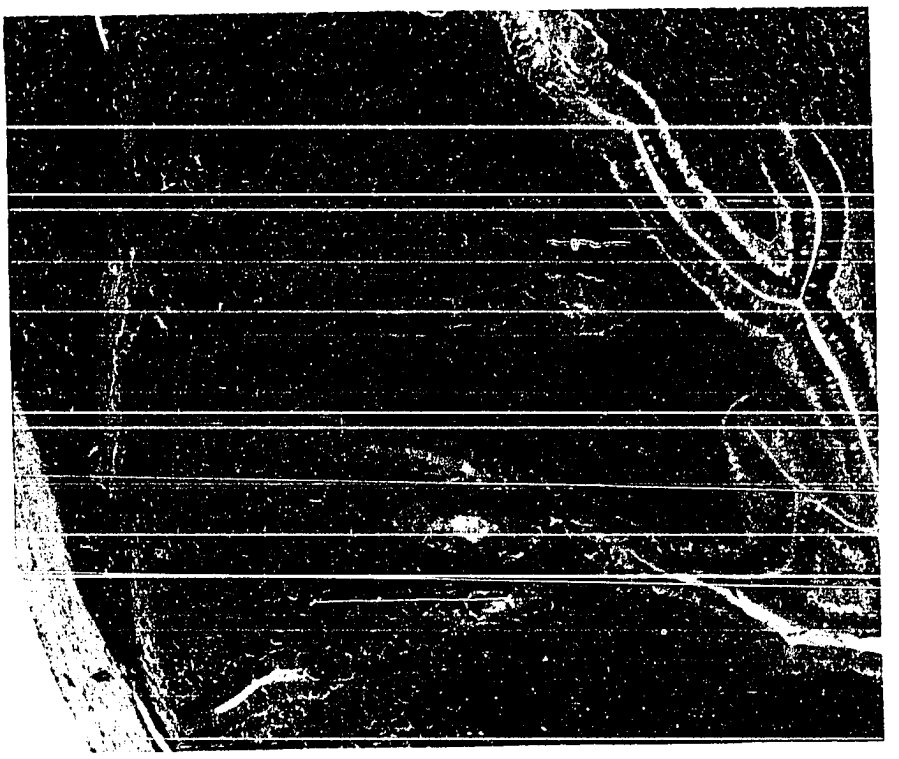
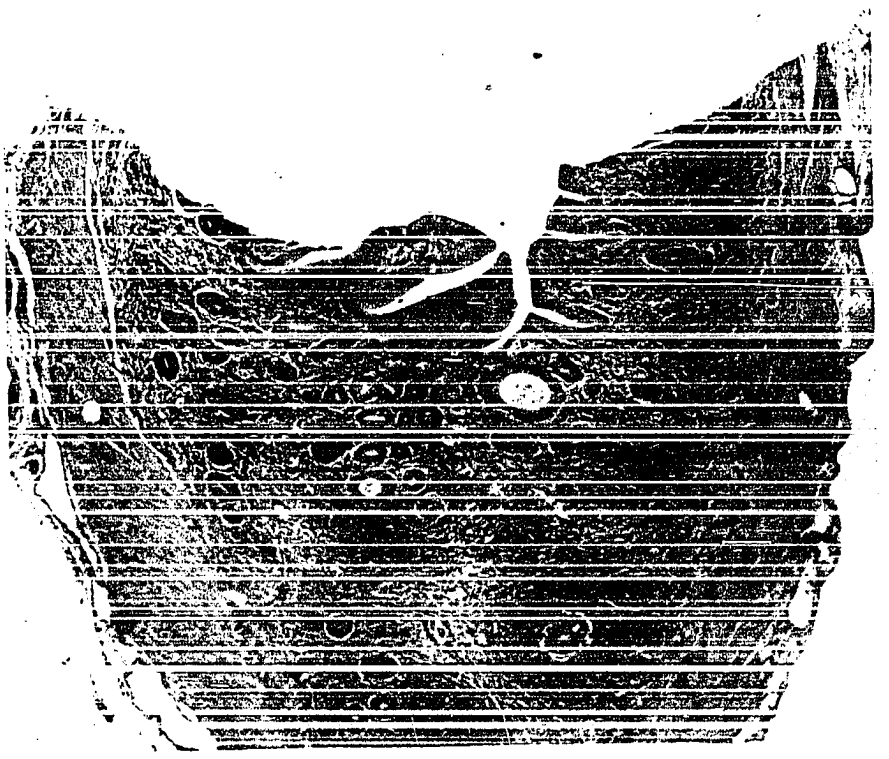


Figure 101. Meckel's diverticulum from a conventional control duck, Trial VI. Large numbers of lymphocytes are present in the submucosa, and lymphoid nodules are adjacent to the muscularis externa. H & E. x100

Figure 102. Meckel's diverticulum from a gnotobiotic control duck, Trial VI. Fewer lymphocytes are present in the submucosa than in the submucosa of the above (Fig. 101) Meckel's diverticulum. H & E. x100



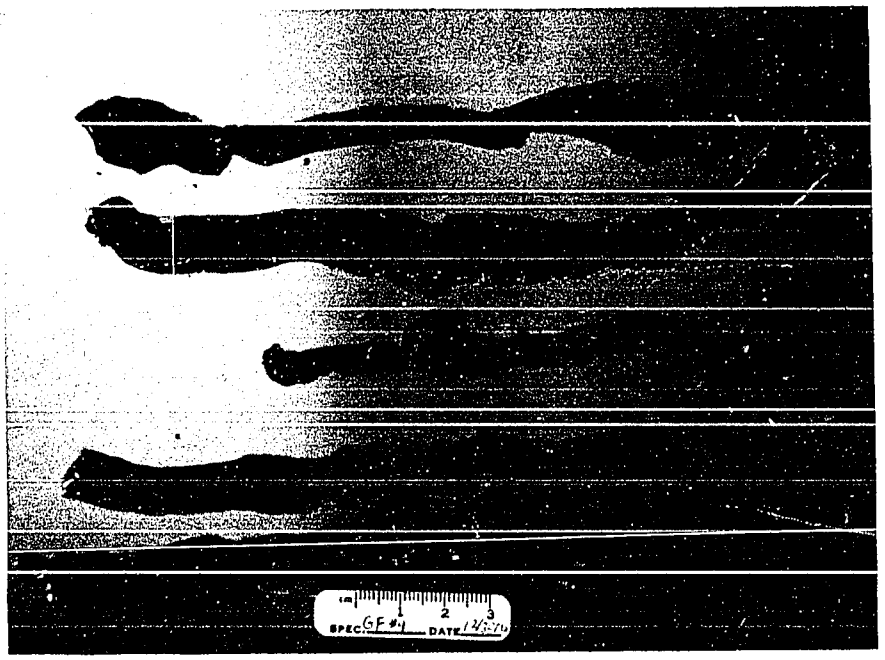


Three gnotobiotic ducks died 3 DPI, and three died 4 DPI. Four of the conventional ducks died 3 DPI, and seven died 4 DPI. Gross lesions were similar in gnotobiotic and conventional ducks. Submucosal petechiae at the esophageal-proventricular junction were covered by diphtheritic plaques. Annular bands in the small intestine were hemorrhagic and visible on the serosal surface. On the mucosal surface they were covered by diphtheritic membranes (Figs. 103 and 104). Scattered submucosal petechiae covered by diphtheritic plaques were present between the annular bands; these submucosal hemorrhages were less numerous in most gnotobiotic ducks. Meckel's diverticulum was hemorrhagic in gnotobiotic and conventional ducks, and a fibrinous plug was present in the lumen (Fig. 103). Submucosal petechiae were present in the ceca, large intestine, cloaca, and bursa of Fabricius (Fig. 105); and tags of fibrin adhered to the mucosal surface in all ducks. Livers were swollen, and the edges bulged on cut surface. Numerous petechiae were present in all livers (Fig. 106), and pinpoint white foci of necrosis were present in the livers of two gnotobiotic and five conventional ducks.

Histologically, lesions of the digestive tract were similar to those described in Trial I. Hydropic degeneration and intranuclear inclusion bodies were present in esophageal and cloacal epithelium (Fig. 107). Diffuse necrosis of lymphocytes and hemorrhage were seen at the esophageal-proventricular

Figure 103. Small intestine from a gnotobiotic duck inoculated with DPV, Trial VI. Two hemorrhagic annular bands covered by diphtheritic membranes are present in the opened sections. Scattered submucosal petechiae are present between the annular bands. Meckel's diverticulum on the unopened section of small intestine is hemorrhagic

Figure 104. Small intestines from a gnotobiotic duck and a conventional duck inoculated with DPV, Trial VI. The two sections of intestine on the left are from a gnotobiotic duck, and the two on the right are from a conventional duck. Annular bands in the conventional and gnotobiotic ducks are hemorrhagic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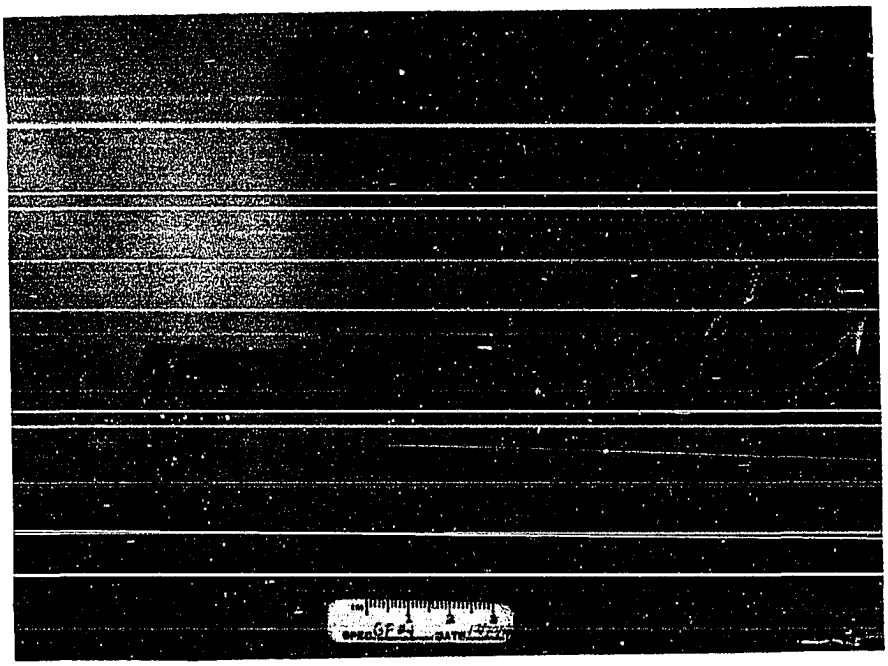
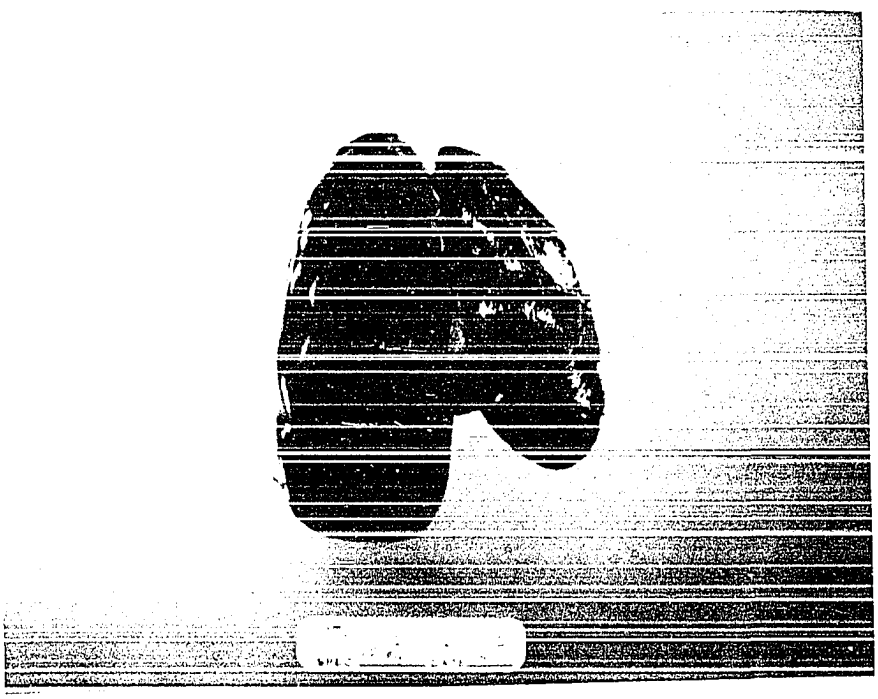
SPEC GF #4
DATE 12/10/71

Figure 105. Terminal ileum, ceca, large intestine, cloaca, and bursa of Fabricius from a gnotobiotic duck inoculated with DPV, Trial VI. The posterior ileal annular band is hemorrhagic; and scattered submucosal petechiae are present in the ceca, large intestine, cloaca, and bursa of Fabricius

Figure 106. Liver from a gnotobiotic duck inoculated with DPV, Trial VI. Multiple petechiae and pinpoint white foci of necrosis are present





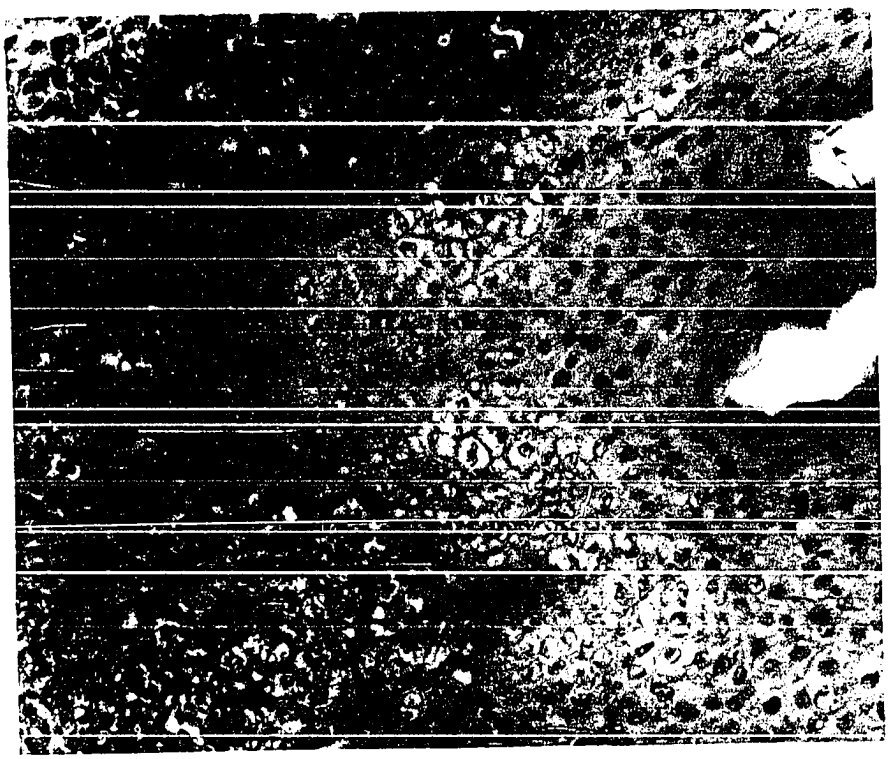
junction (Fig. 108). Fibrocytes, macrophages, and submucosal glands in the esophageal mucosa were degenerate and contained intranuclear inclusion bodies. Diffuse necrosis of lymphocytes was also observed in Meckel's diverticulum (Fig. 109) and in intestinal annular bands (Fig. 110). The overlying mucosa was degenerate, and fibrin was present on its surface. Necrotic fibrocytes were present in the villous lamina propria. Crypt epithelium was degenerate (Fig. 111) and contained intranuclear inclusion bodies.

Multiple nonzonal foci of necrosis and hemorrhage were present in the liver (Fig. 112). Intranuclear inclusion bodies were present in surrounding hepatocytes. Bile duct epithelium was also degenerate, and inclusion bodies were seen in the nuclei (Fig. 113). Scattered foci of necrosis were also present in the pancreas; and acinar, islet, and ductal cells were involved.

Diffuse necrosis of lymphocytes and hemorrhage were observed in the spleen. Reticuloendothelial cells lining the sinusoids and in the periarteriolar reticular sheaths were degenerate or necrotic, and inclusion bodies were present in the nuclei (Fig. 114). Diffuse necrosis of medullary lymphocytes was seen in the thymus and bursa of Fabricius. Lymphocytic depletion and pyknosis were evident in the cortex of the thymus and bursa of Fabricius (Fig. 115). Reticuloendothelial

Figure 107. Esophagus from a gnotobiotic duck inoculated with DPV, Trial VI. Ballooning degeneration is present in the epithelial cells. H & E. x500

Figure 108. Esophageal-proventricular junction from a gnotobiotic duck inoculated with DPV, Trial VI. The crypt of stratified squamous epithelium is degenerate, and some of the epithelial cells have been sloughed into the lumen. Cellular detritus and scattered erythrocytes are all that remains of the submucosal lymphoid nodules. H & E. x500



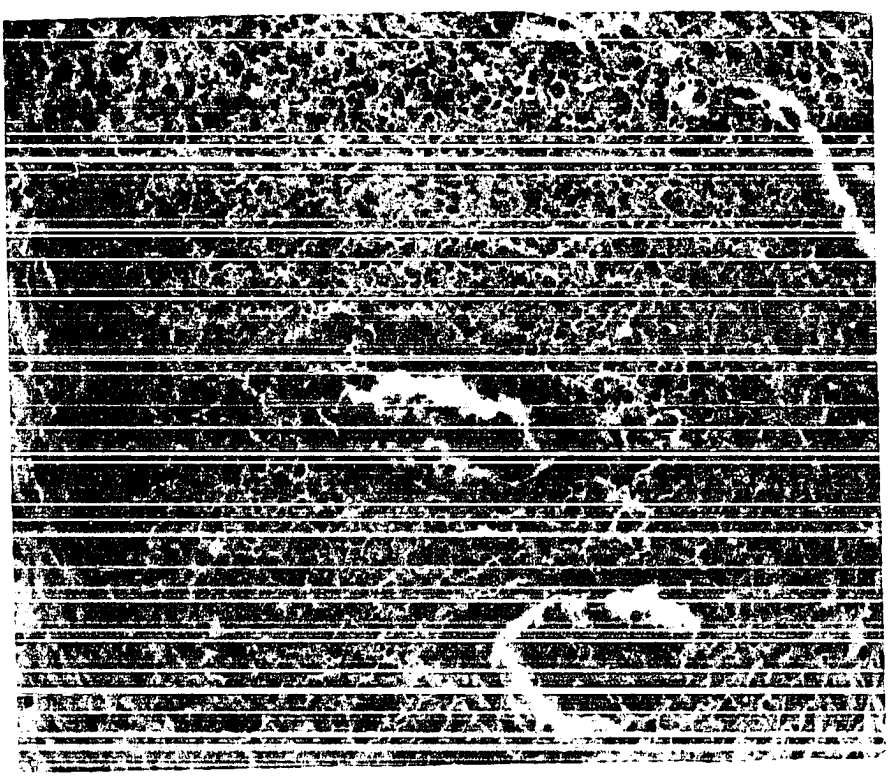


Figure 109. Meckel's diverticulum from a gnotobiotic duck inoculated with DPV, Trial VI. Diffuse necrosis is present in the submucosal lymphoid nodules, and fibrin is present in the lumen of the diverticulum. H & E. x250

Figure 110. Annular band from a gnotobiotic duck inoculated with DPV, Trial VI. An acute diffuse fibrinonecrotic hemorrhagic enteritis is present in the annular band. H & E. x250

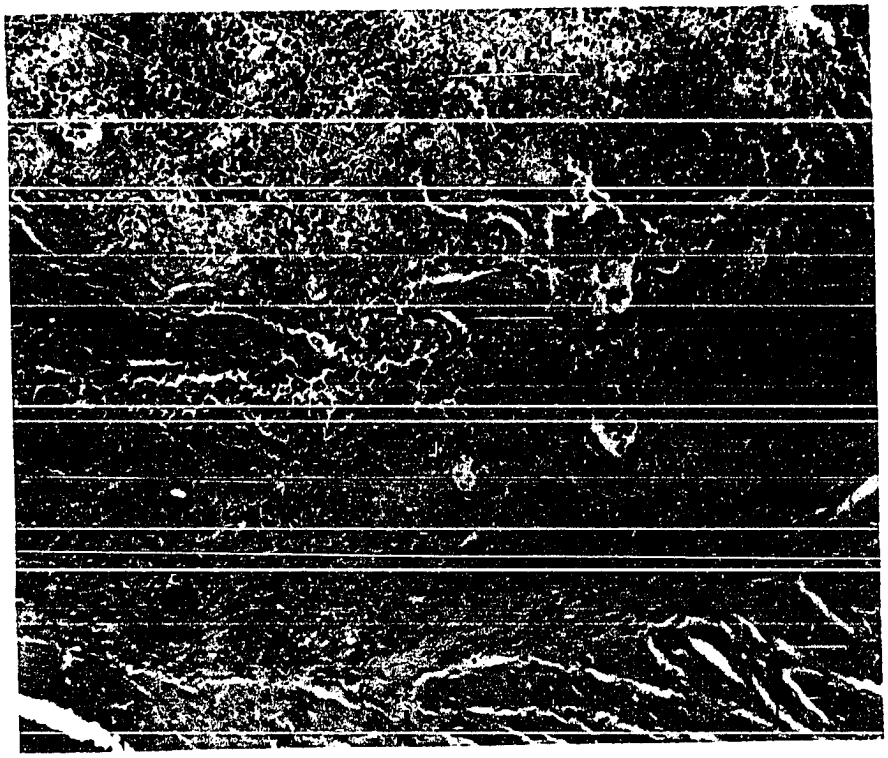
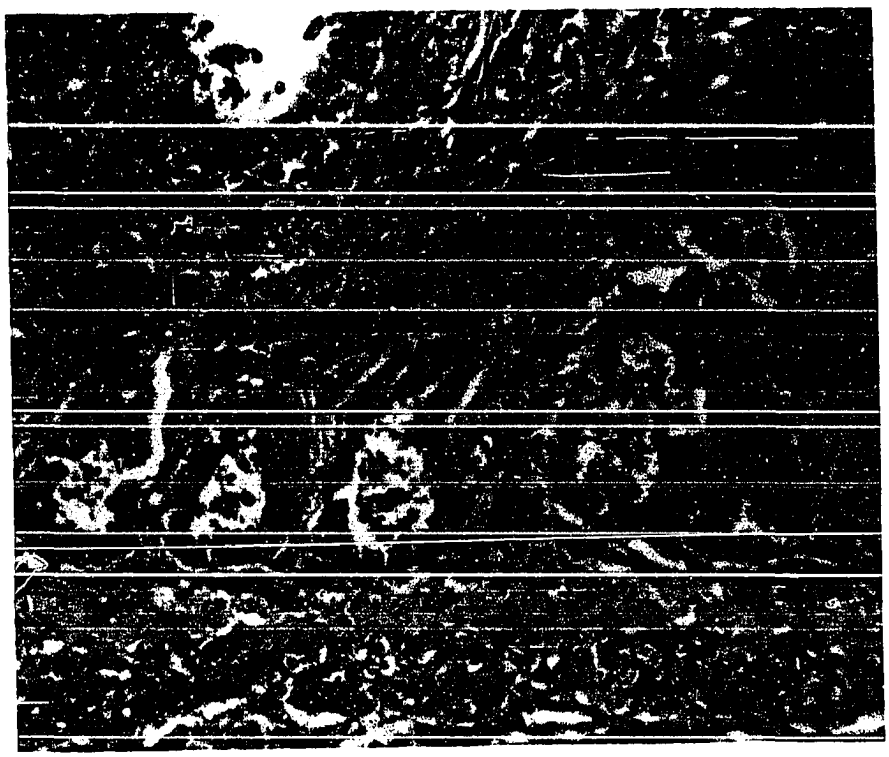


Figure 111. Ileum from a gnotobiotic duck inoculated with DPV, Trial VI. Crypt epithelium is degenerate, and dark nuclear debris is present in the surrounding tissue. H & E. x500

Figure 112. Liver from a gnotobiotic duck inoculated with DPV, Trial VI. Nonzonal focal hepatic necrosis is present. The surrounding hepatic sinusoids are congested. Pyknosis, marginated chromatin, and intranuclear inclusion bodies are present in some of the surrounding hepatocytes. H & E. x900



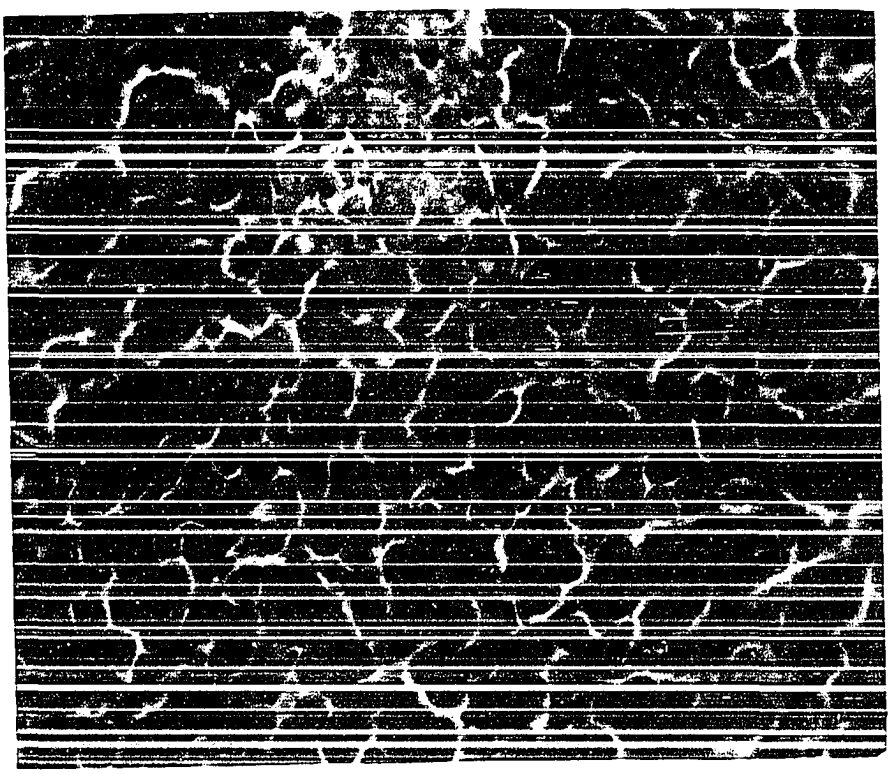
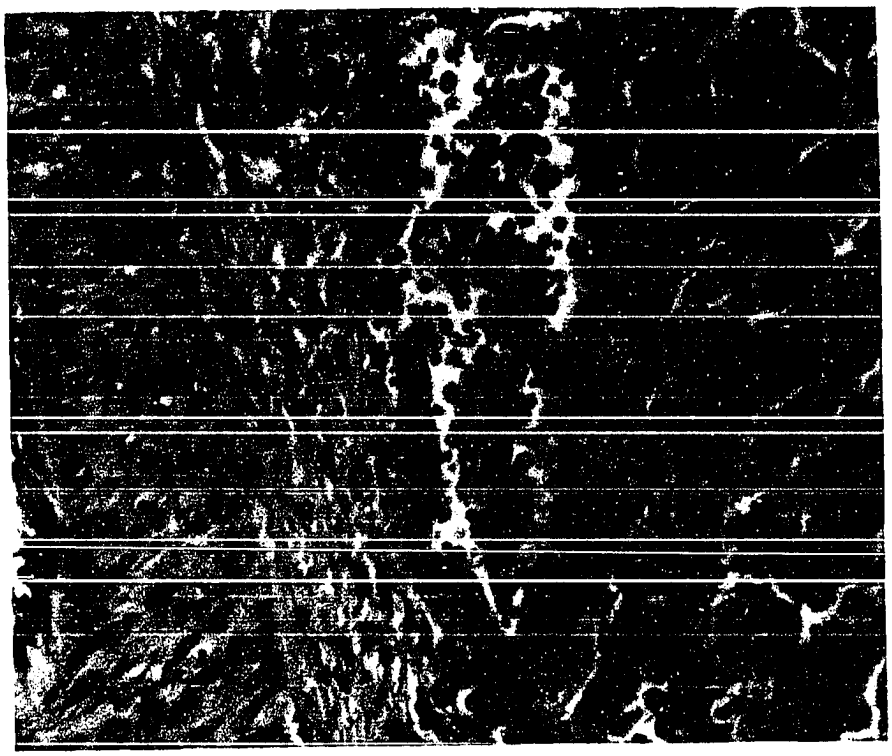
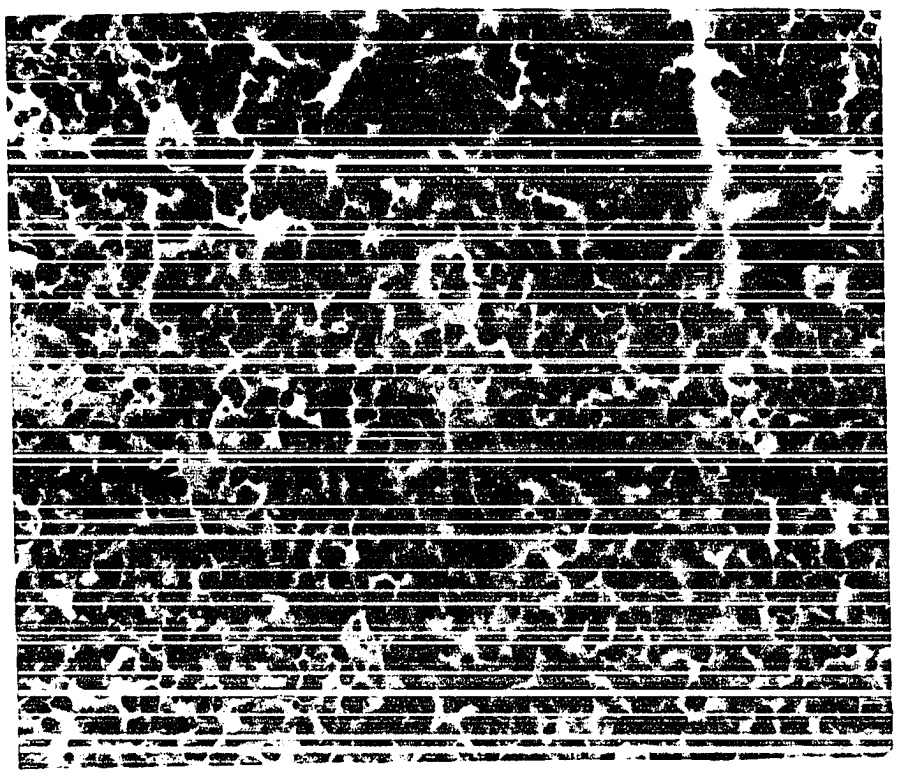


Figure 113. Small bile duct from a gnotobiotic duck inoculated with DPV, Trial VI. Biliary epithelium and surrounding hepatocytes are degenerate and contain intranuclear inclusion bodies. H & E. x900

Figure 114. Spleen from a gnotobiotic duck inoculated with DPV, Trial VI. Diffuse necrosis and hemorrhage are present in the parenchyma. Intranuclear inclusion bodies are present in a few of the reticuloendothelial cells. H & E. x900



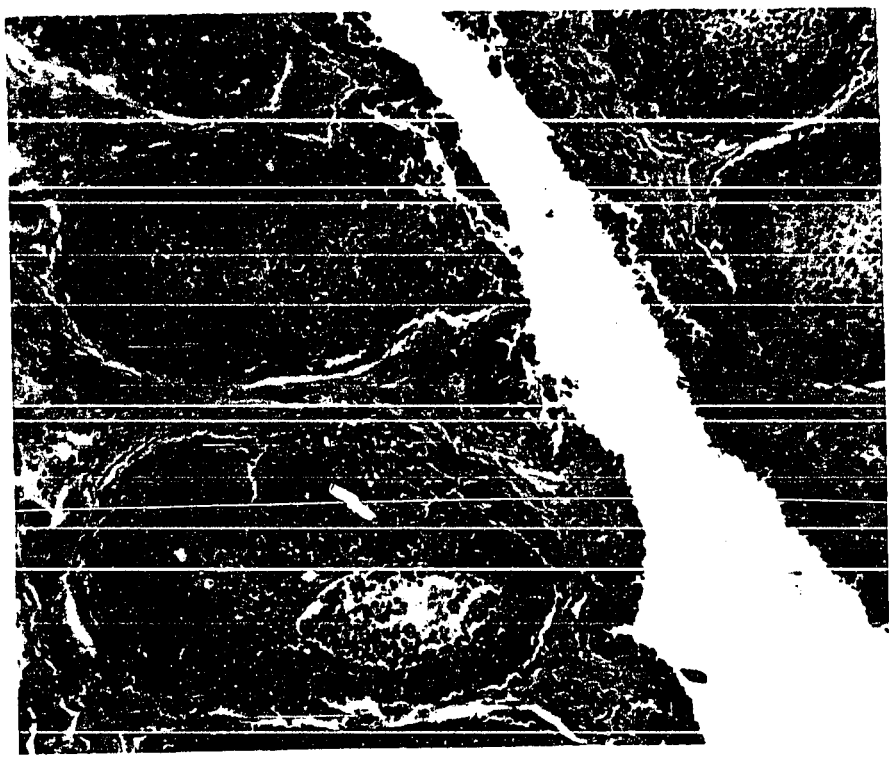


cells in the two organs were degenerate and contained intranuclear inclusion bodies (Fig. 116).

Small foci of degeneration were found in the adrenal cortex, and intranuclear inclusion bodies were present in a few cells. Tracheal epithelium was degenerate, and lymphoid nodules in the peribronchial tissue were necrotic (Fig. 117). Air capillary walls were thickened. Bone marrow cells, osteoblasts lining bony spicules, and osteoclasts were degenerate; and inclusion bodies could be found in a few of these cells. Microscopic lesions were not found in the brain, kidney, and eyes of 4-week-old ducks.

Figure 115. Bursa of Fabricius from a gnotobiotic duck inoculated with DPV, Trial VI. Follicles of the bursa are depleted of lymphocytes. A cystic space containing cellular detritus is present in one follicle. Mucosal epithelial cells lining the bursa of Fabricius are degenerate, and some are sloughed into the lumen. H & E. x250

Figure 116. Higher magnification of one of the above follicles from the bursa of Fabricius, Trial VI. Intranuclear inclusion bodies are present in a few of the reticuloendothelial cells. H & E. x900



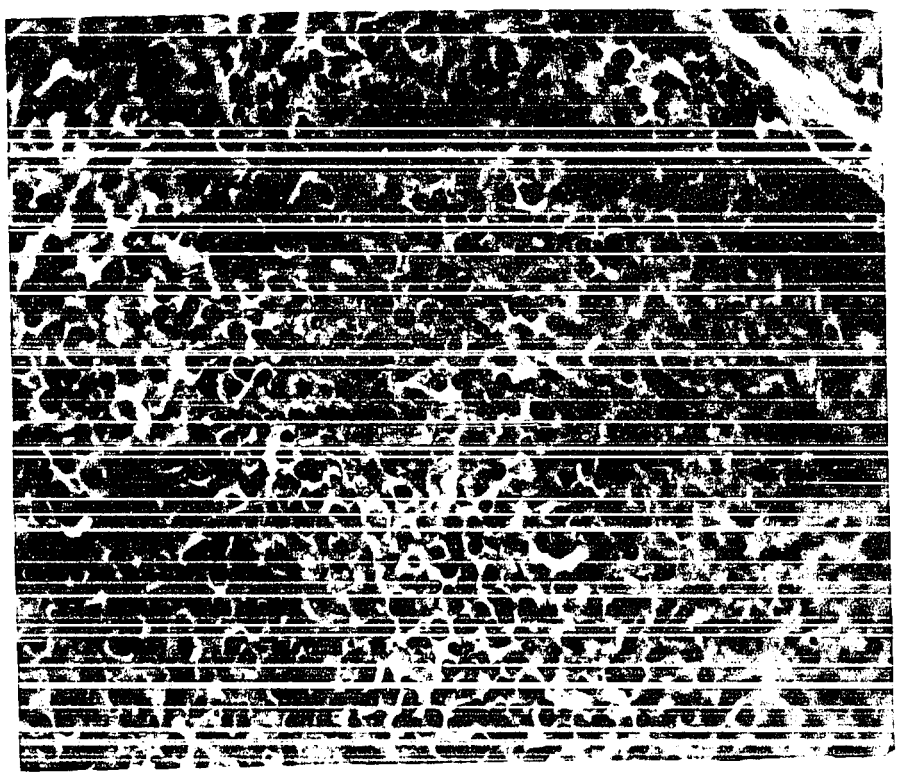
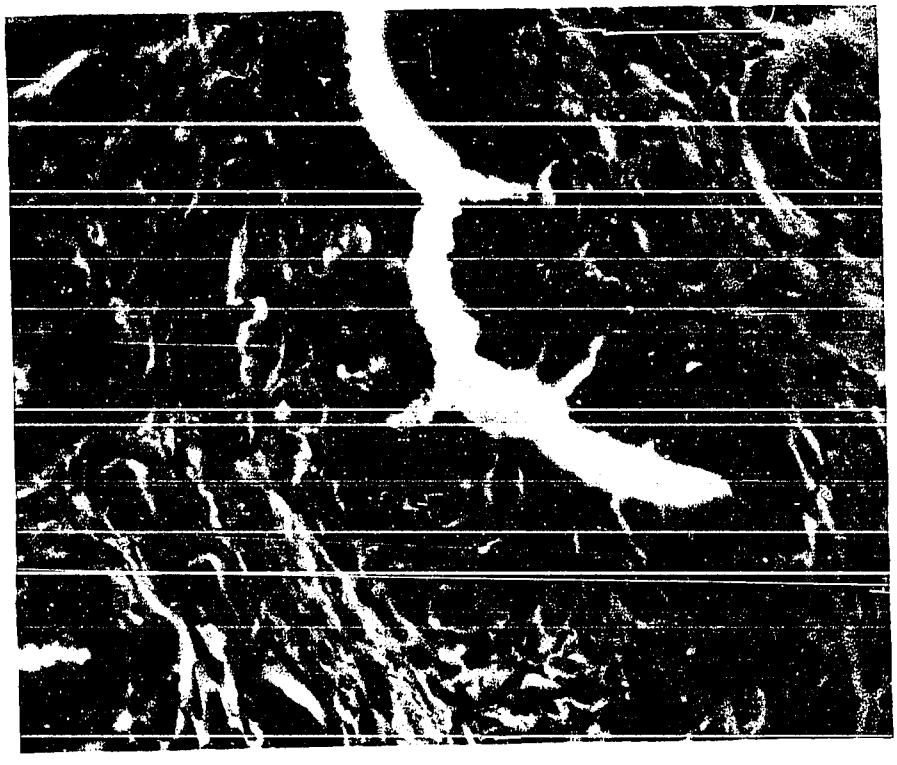


Figure 117. Bronchus from a gnotobiotic duck inoculated with DPV, Trial VI. The bronchial epithelium and the adjacent connective tissue are degenerate, and the vessels are congested. H & E. x900



DISCUSSION

Pathogenesis

Alimentary tract

Small foci of ballooning degeneration in the stratified squamous mucosa were the initial lesions observed in ducks inoculated with DPV. Subsequently, lesions occurred in intestinal crypt epithelium, intestinal villous lamina propria, submucosal lymphoid tissue, fibrocytes, and macrophages. Ballooning degeneration resulted in cytolysis and microvesicle formation in the esophageal and cloacal mucosa. Mucosal vesicles formed by intracellular edema have been reported in a varicella-like disease of monkeys (Blakely et al., 1973) and contrast with vesicle formation initiated by intercellular edema in bovine vesicular stomatitis (Chow et al., 1951; Ribelin, 1958; Seibold and Sharp, 1960). Syncytia were not formed in the basal layers of the esophageal mucosa as reported with herpesvirus-T (Emmons et al., 1968). Esophageal and cloacal diphtheritic membranes were formed by necrosis of the mucosa and adjacent submucosal fibrocytes which allowed plasma to seep through the mucosa and form fibrinous membranes on the mucosa.

Gastrointestinal lesions were located primarily in necrotic lymphocytic tissue at the esophageal-proventricular junction, annular bands, Meckel's diverticulum, and scattered submucosal lymphoid nodules. Although the intestinal lesions

of duck plague grossly resembled "button ulcers" of hog cholera (Dunne et al., 1952; Luedke and Dunne, 1961), vascular endothelial lesions were not present in the intestinal submucosa as postulated by Leibovitz (1971); and vascular lesions were not found in other tissues, for example the brain, as reported in hog cholera (Dunne et al., 1952; Luedke and Dunne, 1961). Neither diffuse submucosal edema nor virus in the submucosal vascular endothelium were found in the duck endothelium as reported in equine viral arteritis (Estes and Cheville, 1970). Swollen endothelium and increased permeability in the submucosal vessels of ducks were probably initiated by histamine-like substances released by necrotic cells. Light and electron microscopic lesions indicated lymphocytic necrosis was the initial lesion in intestinal lymphoid tissue, and hemorrhage occurred as a result of lymphocytic necrosis. Polypeptides and proteases released from lysed cells would potentiate the transitory vascular alterations initiated by histamine. Vascular dilation and stasis of blood flow caused local hypoxia, and subsequently hemorrhage occurred by diapedesis or breaks in devitalized vascular walls.

Necrosis of macrophages and connective tissue in the villous lamina propria and of crypt epithelium occurred throughout the small and large intestines. Necrosis of these cells beneath the basement membrane in the lamina propria was the initial lesion in the intestinal villi. The subsequent contraction of villous smooth muscle, edema in the lamina

propria, and the sloughing of overlying epithelium were similar to lesions produced in rats by compound 48/80 (Fell et al., 1961) and may have been initiated by histamine-like substances released by necrotic cells. Viral antigens or virions were not found in villous epithelium, which would suggest viral growth or multiplication did not occur in villous cells and was not responsible for sloughing of these cells. In contrast, epithelial cells in intestinal crypts, where rapid cell replication occurs, contained virions and large amounts of viral antigens. Degeneration and sloughing of these cells occurred subsequently to viral multiplication. This affinity for undifferentiated epithelial cells and the microscopic lesions produced were similar to those reported in feline panleukopenia (Csiza et al., 1971; Kent and Moon, 1973).

Viral replication in stratified squamous epithelium of the esophagus and cloaca, and in submucosal macrophages and fibrocytes was similar to that described in herpes simplex (Nii et al., 1968) and in fibroblasts infected with DPV (Breese and Dardiri, 1968). Nucleocapsids were seen in the nucleus and partially budded through the inner nuclear membrane into the perinuclear cisternae. Enveloped particles were present in cytoplasmic vacuoles, but nucleocapsids partially budded into cytoplasmic vacuoles as reported in herpes simplex (Nii et al., 1968) were not detected in this study.

Maturation of virions and cellular necrosis were not closely linked in some virus-host systems (Bablanian, 1972;

Smith, 1972), and this was also apparent in the duck intestine. Virions were found in the cytoplasm of fibrocytes, macrophages, and epithelial cells of the esophageal mucosa, cloacal mucosa, and intestinal crypt before cellular degeneration and necrosis occurred. Viral particles in the cytoplasm and nuclei of these cells were similar to those previously observed in duck embryo fibroblasts (Breese and Dardiri, 1968). Cytolysis and karyorrhexis occurred in lymphocytes of the submucosa after nucleocapsids were formed in the nucleus and before very many virions appeared in the cytoplasm.

Liver

Duck plague virus was initially found in hepatic Kupffer cells where viral replication occurred. Previous reports indicated hepatic macrophages were a major pathway for the removal of viruses from the blood and were a primary defense against hepatocytic invasion (Allison, 1974; Mims, 1964; Smith, 1972). For example, in adult mice hepatic macrophages inhibited herpes simplex invasion of hepatocytes (Johnson, 1964). If this macrophage barrier were blocked or destroyed by silica or anti-macrophage serum, hepatitis occurred in adult mice (Hirsch et al., 1970; Zisman et al., 1970). Stevens and Cook (1971) also demonstrated adult mouse macrophages prevented herpes simplex hepatitis in suckling mice. In suckling mice herpes simplex replicated in hepatic macrophages and caused hepatitis. Duck hepatic macrophages, like those of suckling

mice, supported DPV replication and became necrotic. Hepatocytes and bile duct epithelium were invaded and also became necrotic.

Swollen mitochondria with broken cristae, reduplication of the inner nuclear membrane, chromatin margination, and necrosis observed in duck hepatocytes were morphologically similar to those reported in human babies with herpes simplex (Patrizi et al., 1968), and equine fetuses with rhinopneumonitis (Kapp, 1973). Filaments observed in the nuclei of hepatocytes were similar to those reported in chickens inoculated with laryngotracheitis virus (Watrach, 1962) and were different from the coiled strands reported in duck fibroblasts inoculated with DPV (Breese and Dardiri, 1968). The three types of DNA cores (rod, dense sphere, and hollow sphere) observed in the hepatocytic nuclei of the duck were similar to those described in herpes simplex (Nii et al., 1968).

Although bile duct lesions were reported in the equine fetal liver with rhinopneumonitis, viral replication was not reported in the biliary epithelium (Kapp, 1973). In the biliary epithelium of the duck, DPV replicated and caused lesions similar to those observed in hepatocytes.

Spleen, thymus, and bursa of Fabricius

Duck plague virus localized first in reticuloendothelial cells of the spleen, thymus, and bursa of Fabricius. In the chicken it has been reported that particles larger than 2 to

3 μm localized in reticuloendothelial cells of splenic red pulp and particles smaller than 2 μm were removed by reticuloendothelial cells of the periarteriolar reticular sheaths (White and Gordon, 1970). Although no attempt was made to detect viremia in these studies, the early appearance of virions in the periarteriolar reticular sheaths (Schweigger-Seidel sheaths) suggested the presence of a viremia, a part of which was not associated with circulating leukocytes. Intranuclear inclusion bodies in these cells contained nucleocapsids. Nucleocapsids in the cytoplasm of macrophages and lymphocytes may have been phagocytized or escaped through breaks in the nuclear membranes.

Lymphocytic necrosis in the bursa of Fabricius, thymus, and spleen of the duck was similar to that reported in acute irradiation of mice lymph nodes (Smith et al., 1967) and Gumboro disease of chickens (Cheville, 1967). Viral particles or antigens were not found in degenerate lymphocytes in Gumboro disease of chickens (Cheville, 1967) suggesting a toxic viral product such as the penton of adenovirus (Ginsberg et al., 1966) may be present in Gumboro disease virus. In duck plague, viral antigens and nucleocapsids were found in the lymphocytes before necrosis occurred. Necrosis of lymphocytes may have been caused by the accumulation of a toxic viral product (Bablanian, 1972) or by activation of lysosomal enzymes by the virus (Allison, 1967). Two types of lysosomes have been described in lymph nodes: L15 lack hydrolases and

are present in lymphocytes; and L19 contain hydrolases and are present in macrophages (Bowers and de Duve, 1967).

Necrotic reticuloendothelial cells in the bursa of Fabricius, thymus, and spleen of ducks contained virions in cytoplasmic vacuoles. Although some phagocytosis of degenerate lymphocytes was seen, reticuloendothelial hyperplasia as reported in Gumboro disease was not observed in duck plague. Cytopathic effect in these cells like that of poliovirus-induced cytopathology (Bablanian et al., 1965a, 1965b) was related to the synthesis and accumulation of viral proteins. Inhibitors of protein synthesis such as puromycin and cycloheximide would probably inhibit the cytopathic effect of duck plague virus as reported for other viruses (Bablanian, 1968, 1970). The difference in the rate of necrosis observed in reticuloendothelial cells and in lymphocytes of the bursa of Fabricius, spleen, and thymus may be due to the rate of accumulation of cytotoxic viral products in the two cells (Bablanian, 1972) or possibly to the presence of different lysosomal content in the two cells (Bowers and de Duve, 1967).

Other organs

In the duck, DPV caused necrosis of tracheal and bronchial epithelium and submucosal lymphoid nodules similar to those reported in laryngotracheitis in chickens (Purcell, 1971), pseudorabies in swine (Baskerville, 1972; Kluge and Marê, 1974), bovine rhinotracheitis (Chia and Savan, 1974),

and feline rhinotracheitis (Crandell et al., 1961). Occasional syncytia were found in the peribronchial tissue of ducks similar to those reported in laryngotracheitis of chickens (Purcell, 1971). Lipoproteins produced by cells infected with herpesviruses have been reported to cause giant cell formation (Tokumaru, 1968). Thickening of the duck air capillary walls was similar to the interstitial pneumonia observed in pseudorabies in swine (Baskerville, 1972), and canine herpesvirus infection (Percy et al., 1971; Thompson et al., 1972). Intranuclear inclusion bodies in all of these cells suggested DPV was responsible for these lesions.

Intranuclear inclusion bodies and viral antigens in cells of the pancreas, ureters, adrenal gland, liver, bursa of Fabricius, thymus, spleen, lung, and intestinal mucosa indicated the pantropism of DPV. Vascular lesions similar to those reported in equine viral arteritis (Estes and Cheville, 1970) or endothelial proliferation as reported in Newcastle disease (Cheville et al., 1972) were not seen in ducks inoculated with DPV. However, necrosis of cells was observed in the parenchymatous tissue that appeared to initiate hemorrhage as described in the digestive tract.

Neurological signs observed in ducks (Leibovitz, 1972) could not be explained because microscopic lesions were not found in the central nervous system. Duck plague virus was not found in cerebral vascular endothelium as observed in Newcastle

disease (Cheville and Beard, 1972), and this lack of endothelial destruction may have prevented DPV from crossing the blood-brain barrier. Electrolyte imbalance caused by digestive tract lesions probably initiated the neurological signs observed in duck plague (Kent and Moon, 1973).

Effect of Lead on the Susceptibility of Ducks to Duck Plague Virus

Blood lead levels in Trial IV initially rose and then declined. The decline could represent a compensatory rate of excretion by the kidney, a decreased rate of absorption by the digestive tract, or a combination of decreased absorption and increased excretion. Low levels of lead in kidney and liver with a higher level in the bone in both Trials IV and V indicated ducks were exposed to lead over a long period of time (Buck et al., 1973).

Trial IV indicated the ingestion of lead shot increased the susceptibility of ducks to DPV. Two hypotheses could explain the results of this trial: (1) Low levels of lead may have inhibited interferon production in ducks as previously reported in mice (Gainer, 1973), and this decreased production of interferon increased the susceptibility of ducks given lead shot to DPV. Although interferon has been reported to inhibit replication of herpes viruses (Lodmell and Notkins, 1974), Vengris (1973) was unable to demonstrate that low levels of

lead inhibited interferon production in chickens. (2) Low levels of lead may have inhibited phagocytosis of DPV by reticuloendothelial cells, allowing the virus to invade parenchymal cells in the early stages of viremia and cause death. Blockage of the reticuloendothelial system in adult mice destroyed their resistance to herpes simplex, and hepatitis occurred in adult mice (Hirsch et al., 1970; Zisman et al., 1970).

The results of Trial V did not support the hypothesis that low levels of lead increased the susceptibility of ducks to DPV. Taking the results of Trials IV and V and combining them, it was not possible to conclude that low levels of lead increased the susceptibility of ducks to DPV. Two different procedures were used in Trials IV and V which may partially explain the different results obtained. (1) Five ml of blood were collected weekly from each duck in Trial IV after lead shot were given orally, but this was not done weekly in Trial V. This blood loss may have decreased the resistance of ducks on low levels of lead. (2) Ducks in Trial IV were given more DPV orally (0.5 ml of 10^4 $DELD_{50}$ per ml) than ducks in Trial V (10^2 $DELD_{50}$). Although the phagocytic capacity of reticuloendothelial cells was not determined in this experiment, the larger dose of virus in Trial IV may have overwhelmed the impaired reticuloendothelial cells in ducks given lead shot. The smaller dose of virus given in Trial V may not have

overwhelmed the reticuloendothelial system and allowed the disease to progress simultaneously in control ducks and those given lead shot.

Gnotobiotic Ducks

Duck plague virus, like feline infectious peritonitis virus (Wolf and Griesemer, 1966), feline rhinotracheitis virus (Hoover et al., 1970), and canine hepatitis virus (Griesemer, 1968), produced a similar disease in respective gnotobiotic and conventional hosts. In the absence of Salmonella and Pasteurella thought to potentiate duck plague epidemics (Dardiri, 1975), nonzonal hepatic necrosis was observed in gnotobiotic ducks inoculated with DPV. These degenerate hepatocytes contained intranuclear inclusion bodies similar to those observed in conventional ducks (Trial III) which contained virions. Tracheal lesions in ducks were also similar to those produced in germfree cats by rhinotracheitis virus, emphasizing that virulent herpesviruses can produce disease without the synergistic action of bacteria.

Lesions of the digestive tract in gnotobiotic ducks were similar to those in conventional ducks after inoculation with DPV. Although the intestinal lymphoid tissue of gnotobiotic ducks was not as well developed as it was in conventional ducks, gross and microscopic lesions of duck plague were similar in conventional and gnotobiotic ducks. Intestinal

lymphoid tissue was sufficiently developed in day-old conventional ducks to initiate lesion development after inoculation with DPV (Erickson et al., 1974). Unlike feline infectious enteritis virus which did not cause lesions in crypt epithelium in the germfree cat (Rohovsky and Griesemer, 1967), DPV caused lesions in the intestinal crypts of gnotobiotic ducks similar to those observed in conventional ducks. It has been suggested that the sluggish proliferation of crypt epithelium in germfree cats was responsible for the crypt epithelial resistance to feline infectious enteritis virus (Kent and Moon, 1973), and it was not determined if the proliferation of crypt epithelium was equal in germfree and conventional ducks.

Since death occurred as rapidly in conventional as in gnotobiotic ducks, bacteremia or bacterial toxemia were not considered necessary for DPV to cause disease in and kill ducks. Bacteremia was no more frequent in irradiated mice with the intestinal syndrome than in nonirradiated control mice (Kent et al., 1968), indicating bacteremia was not necessarily a sequela to intestinal denudation, since death occurred as rapidly after intestinal denudation by irradiation in germfree as in conventional mice (Kent et al., 1968; Matsuzawa and Wilson, 1965); absorbed bacterial toxins were also not a major factor in death after intestinal denudation. Irradiation of 1,000 R destroys primarily crypt epithelium

similar to that observed in ducks inoculated with DPV and also kills mice in 3 to 6 days.

Several physiological factors probably contributed to the diarrhea observed in ducks inoculated with DPV. The destruction of crypt epithelium and sloughing of the villous epithelium destroyed the absorptive capacity of the intestine. Thus, the intestine would be unable to absorb the ingesta or the electrolyte-rich alimentary excretions. Removal of the mucosal barrier would allow fluid and electrolytes to move into the lumen of the intestine with a decreased absorptive capacity and an increased rate of motility. Lesions in the large intestine where absorption of fluids occurs probably also contributed to the diarrhea observed clinically in ducks.

SUMMARY

White Pekin ducks were inoculated with duck plague virus (DPV) isolated from a mallard duck during the 1973 epidemic at Lake Andes, South Dakota. Tissues were collected sequentially from the digestive tract, spleen, thymus, bursa of Fabricius, and liver; and each was examined by light, electron, and fluorescent microscopy.

Small foci of ballooning degeneration in the stratified squamous mucosa were the initial lesions of the alimentary tract observed after ducks were inoculated with DPV. Subsequently, lesions occurred in esophageal submucosal glands, intestinal villous lamina propria, and submucosal lymphoid tissue. Viral antigens and virions were detected in degenerate or necrotic fibrocytes, macrophages, esophageal submucosal glandular and mucosal epithelium, and intestinal crypt epithelium. In contrast, degenerate or necrotic lymphocytes in annular bands or Meckel's diverticulum contained viral antigens and large numbers of nucleocapsids, but few virions.

Twenty-four hours later DPV had localized in the reticuloendothelial system. Reticuloendothelial cells of the spleen, thymus, and bursa of Fabricius contained large amounts of viral antigens, and virions were observed in the cytoplasm before the cells became necrotic. Medullary lymphocytes of the thymus and bursa of Fabricius were more severely affected than cortical lymphocytes, and both the T and B lymphocytes

were affected in the spleen. Although lymphocytes in all three organs contained viral antigens and nucleocapsids, necrosis occurred before virions were formed in the cytoplasm of most lymphocytes.

In the liver, DPV was also found first in Kupffer cells where viral replication occurred. Hepatocytes and biliary epithelium were subsequently invaded. Degeneration and necrosis of Kupffer cells, hepatocytes, and biliary epithelium occurred after virions were found in the cytoplasm. Intranuclear inclusion bodies observed histologically in hepatocytes, biliary epithelium, and cells of the alimentary tract, bursa of Fabricius, spleen, and thymus, were formed ultrastructurally by fine granular material in the center of the nuclei which contained nucleocapsids and marginated chromatin.

Histologic lesions also were observed in the respiratory tract, kidney, adrenal gland, heart, and bone marrow. There was necrosis of tracheal and bronchial epithelium and interstitial pneumonia in the respiratory tract, and intranuclear inclusion bodies and viral antigens were present in these cells. Foci of ureteral epithelium, pancreatic acinar and islet cells, and adrenal cortical cells were degenerate and necrotic; and they also contained intranuclear inclusion bodies and viral antigens. Hemorrhage was frequently present in these foci of parenchymal degeneration. Lymphoid nodules

were necrotic in all tissues examined and contained viral antigens.

In one experiment, lead shot given orally appeared to increase the susceptibility of ducks to DPV, but a second experiment failed to support this conclusion. Gross and histologic lesions of duck plague were similar in ducks given lead shot and control ducks.

Gnotobiotic ducks (with only Escherichia coli and Streptococcus faecalis in the isolator) were inoculated with DPV. Similar death rates and gross and microscopic lesions were observed in gnotobiotic and conventional ducks. The synergistic action of bacteria or bacterial toxins was not necessary for DPV to cause cellular necrosis in the alimentary tract and parenchymatous organs such as the liver, spleen, thymus, bursa of Fabricius, and lung.

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